Conference Paper

# Specific Primer for Human Bcl-2-Protein Expression Analysis

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## Abstract

Bcl-2 protein (B-cell lymphoma 2) is one of the proteins in the Bcl-2 protein family that are anti apoptotic and encoded by the BCL-2 gene. This protein controls the permeability of the outer membrane of the mitochondria, which plays a role in the release of cytochrome c and several other proteins that contribute to cell death. Bcl-2 protein plays a role in regulating cell death and regulating the balance between new and old cells. Several studies showed that changes in the structure and function of Bcl-2 play a role in cell apoptosis disorders, producing cancer cells. High expression of Bcl-2 protein is seen in lung, breast, lymph node and brain cancers. Analysis of Bcl-2 protein expression at the molecular level in cancer cell is needed to determine the expression level of this protein. This study aims to produce the Bcl-2 primer for qPCR method that specifically identifies the target gene, and can be used to see the BCL-2 gene expression level in cells. A BCL-2 sequence was obtained from the National Center for Biotechnology Information (NCBI). The next process is further analysis with Primer-BLAST (NCBI). Some primer candidates are produced and undergo another selection based on several criteria. Finally, one pair of potential primers was obtained for the reaction of qPCR to BCL-2. Primer specificity was tested with Basic Local Alignment Tools (BLAST) tools and result in specific primer for human BCL-2 gene. Annealing temperature optimization shows that the primer can react optimally at 59°C.

**Keywords:** Bcl-2 protein, BCL-2 gene, qPCR, primer, gene expression, apoptosis

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## 1. Introduction

In this modern era, bioinformatics methods are in high demand in the field of molecular biology. Primer design is one of bioinformatics method used for Polymerase Chain Reaction (PCR) techniques. Primers are nucleotide bases which generally have 18-22 base pairs useful for initiating the process of DNA synthesis. The primer must be specific to the template that is used.

Bcl-2 (B-cell lymphoma 2) is a regulatory protein that regulates cell death by inhibiting apoptosis (anti-apoptosis)123. Apoptosis is a biological mechanism that maintains the balance of cells in the body. The Bcl-2 expression level is seen to increase in some cases of cancer, including melanoma, breast, prostate, chronic lymphocytic leukemia,

and lung cancer 456. It also likely becomes a factor in autoimmunity prevention 7. This protein has become a research concern in cancer therapy and detection 891011.

Gene expression level can be used as a marker the role of a gene in the incidence of disease. The quantitative real time PCR (qPCR) method is generally used to calculate gene expression levels, in absolute value or relative to standard proteins. To do this, the qPCR method needs a primer that specifically recognizes the target gene.

This study design a primer for BCL-2 gene in human samples using some tools. This primer will be used in qPCR using intercalating dyes (SYBR green) to measure the BCL-2 gene expression level in human cells.

## 2. Methods

### 2.1. BCL-2 Gene Data

The BCL-2 gene sequence was obtained from the NCBI (National Center for Biotechnology Information) site with accession number KY098799.1. This sequence had a length of 870 bp. Exon position was found in bases 286 to 870. FASTA sequence files were then downloaded and used for the primer design process.

### 2.2. Design Primer Using Primer-BLAST Tools

Primer-BLAST tools were used to design primers according to the desired gene target. The BCL-2 gene sequence that had been obtained previously was then entered into the system. Exon/intron selection was not carried out. Some candidate primers obtained then underwent further selection such as selection in forward and reverse primer temperatures <5°C, % GC values at 40-60% and no self-3-complementarity values. The specificity of this primer was tested using Nucleotide BLAST (Basic Local Alignment Searching Tools).

### 2.3. Annealing Temperature Optimization for the Primer

Optimization was done to see optimum temperature needed for amplification process. Here, we use RNA from HeLa cells. The reference gene was the 18S gene with primer from other publications12. The reagent used was SensiFAST SYBR No-ROX One Step Kit (Bioline, Canada). The reaction mixture used was: 2x SensiFAST $^{TM}$ SYBR® No-ROX One-Step Mix = 10 ul, 10 uM Primary Forward = 0.8 ul, 10 uM Primary Reverse = 0.8 ul,

Reverse Transcriptase = 0.2 ul, RiboSafe RNase Inhibitor = 0.4 ul, ddH2O = 3.8 ul and RNA Template = 4 ul. The temperature cycle used was 45°C for 10 minutes, 95°C for 2 minutes, 95°C for 5 seconds, gradation of temperature 55°C - 65°C for 10 seconds and 72°C for 5 seconds on the CFX Connect machine (BioRad, USA) 13.

## 3. Result

Using the bioinformatics tools, we obtained 6 primer candidates with different characteristics. Further selection of these primers, produced 1 potential primer pair (Table 1).

TABLE 1: Potential Bcl-2 Primers.

| | Primer | Suhu Tm | % GC | Self 3' Complementary |
|---|---|---|---|---|
| Forward | CTGGGAGAACGGGGTACGAT | 61,4°C | 60 | 2 |
| Reverse | GACCCCACCGAACTCAAAGA | 59,6°C | 55 | 1 |

The relative position of the Bcl-2 primer to BCL-2 gene is at the 504-523 base position for forward primer and 961-942 base for the reverse primer. The DNA length will be produced is 458 bp. The primary position picture is shown in Figure 1

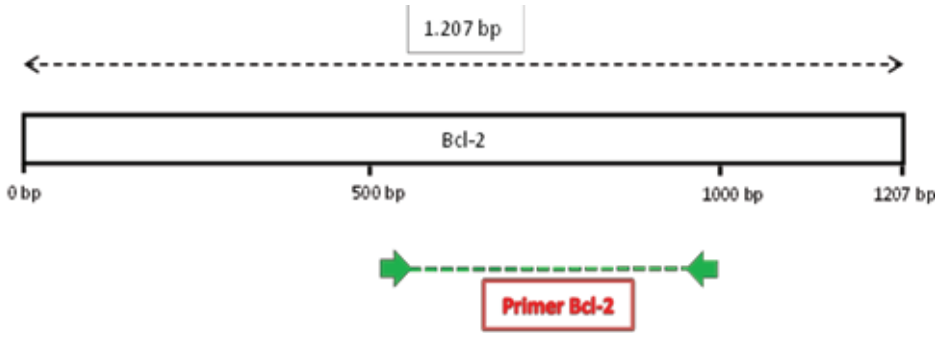


**Figure** 1: The relative position of the Bcl-2 primer to Bcl-2 gene. The primary pair (arrow) is located between 500-1000 bases. The amplification result is 458 bp.

Primer specificity test with BLAST showed in table 2. The level of similarity and E-value for the Bcl-2 primer sequence to other DNA sequences showed that Bcl-2 primer was specific in recognizing the human BCL-2 gene.

The results of the annealing temperature optimization are shown in Figure 2. This figure shows that the Bcl-2 primer can provide optimal results at the temperature of 59°C. At this temperature, the Ct value (threshold cycle) is less than other annealing temperatures. Reference genes can also be amplified at this temperature.

Table 2: BLAST Result of Bcl-2 Primer.

| Primer | No. Aksesi | Sekuen | Tingkat Kesamaan | E-value |
|---|---|---|---|---|
| CTGGGAGAACGGGGTACGAT | KY098818.1 | Homo sapiens isolate PB605 BCL2 (BCL2) gene, exon 2 and partial cds | 100% | 0,017 |
| | KY098817.1 | Homo sapiens isolate EP103 BCL2 (BCL2) gene, exon 2 and partial cds | 100% | 0,017 |
| | KY098814.1 | Homo sapiens isolate CB204 BCL2 (BCL2) gene, exon 2 and partial cds | 100% | 0,017 |
| GACCCCACCGAACTCAAAGA | KY098818.1 | Homo sapiens isolate PB605 BCL2 (BCL2) gene, exon 2 and partial cds | 100% | 0,017 |
| | KY098817.1 | Homo sapiens isolate EP103 BCL2 (BCL2) gene, exon 2 and partial cds | 100% | 0,017 |
| | KY098816.1 | Homo sapiens isolate CB210 BCL2 (BCL2) gene, exon 2 and partial cds | 100% | 0,017 |

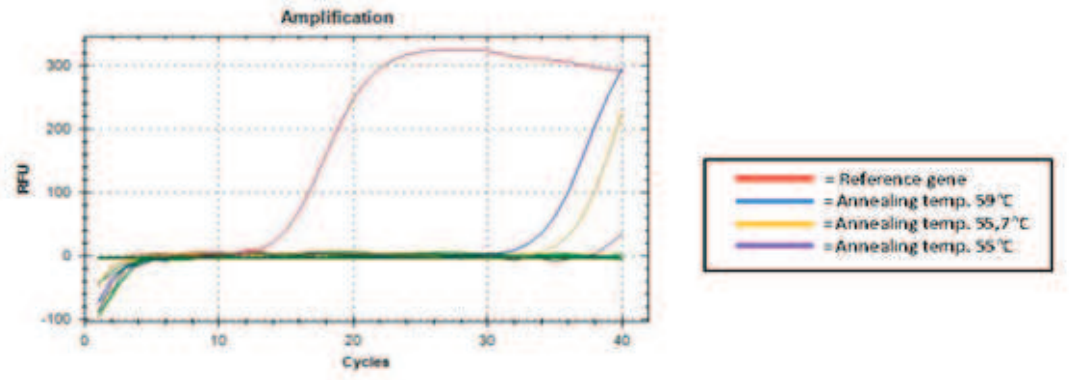


**Figure** 2: The results annealing temperature optimization. Visible optimal amplification results are seen at annealing temperature of 59°C.

## 4. Discussion

The qPCR (quantitative PCR) is a method of DNA propagation using RNA samples. This RNA molecule is reverse transcribed to cDNA, so qPCR is, in actuality, a short term of RT-PCR (Reverse Transcription Quantitative PCR). This method is widely used in several research topics including gene expression. The advantage of the qPCR method compared to other methods is that despite the small number of RNA samples, it can produce huge amount of data in short time 14. One of the dyes used in qPCR is intercalating dyes, SYBR green. This dye is often used in the qPCR method because of

economical reason. However, the use of SYBR green in qPCR requires a specific primer design because SYBR green will recognize all double-strand DNA formed 14.

One of the main aspect that has to be considered in qPCR method is the primer. Primer is small nucleotides sizes, around 18-24 bp, for DNA amplification. These primers must be designed so that they can specifically identify the target gene. Some criteria must be met by these primers in order to function properly, including (i) the% GC value at 40-60%, (ii) the temperature difference between primer pairs not more than 5˚C, (iii) does not form a dimer primer and (iv) does not form hairpin. A good primer is not solely based on the primer characteristics, but also on the condition of the target genes, such as its uniqueness, location and structure 15.

This study has produced Bcl-2 primers that specifically can recognize the BCL-2 gene in humans. Annealing temperature optimization of Bcl-2 was also carried out. This optimization is important to see the primer ability to attach to the target gene. So that multiplication of genes can be done. This is the reason that annealing temperature value (Ta) of a primer requires more attention than the melting temperature (Tm) 15. This optimization results that Bcl-2 primer can react optimally at 59˚C. Good optimization and validation of the primers will give a good results and in addition will save time and costs as well as optimizing using precious samples 15.

This Bcl-2 primer can be used for BCL-2 gene expression study in humans related to its function in cell apoptosis. Further optimization needs to be done for this primer, related to its use in the health sector. The optimization will improve the quality of the data obtained, it can be trusted and published properly 16.

## 5. Conclusion

This study succeeded in designing Bcl-2 primers under certain criteria. The collection of BCL-2 gene database was carried out from NCBI which then processed further with Primer-BLAST. The comparison of Bcl-2 primer sequence with other sequences with BLAST result that the Bcl-2 primer was specific to the BCL-2 gene in humans. Optimization of the annealing temperature on the Bcl-2 primer showed that this primer works optimally at 59˚C. Further optimization needs to be done to use Bcl-2 primer in gene expression analysis.

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## Conflict of Interest

The authors declare that there are no conflicts of interest with respect to the research, authorship and/or publication of this article.

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