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# Purification of 3×Myc PKG-Puro-Poly A Gene for Bacterial Transformation

**Khairil Pahmi[1*], M. Sidratullah[2]**

[1] Medical Education Study Program, Faculty of Medicine, Mataram University
Jl. Pemuda No.37, Gomong, Kec. Selaparang, Kota Mataram, Nusa Tenggara Barat, Indonesia

[2] Pharmacy (DIII) Study Program, Faculty of Health Science, Nahdlatul Wathan Mataram University
Jl. Merdeka Raya Karang Pule Kec. Sekarbela Kota Mataram, Nusa Tenggara Barat Indonesia

* Corresponding Author. Email: khairil_pahmi@staff.unram.ac.id

## ABSTRAK

Disregulasi onkoprotein c-Myc (Myc) terlibat dalam banyak jenis kanker. Myc adalah faktor transkripsi spesifik urutan yang mengatur transkripsi gen yang terlibat dalam pengendalian proliferasi sel dan apoptosis melalui mekanisme yang belum dipahami dengan baik. Metode penelitian ini adalah eksperimental. Hasil percobaan akan dijelaskan. Beberapa proses akan dilakukan dengan cara Polymerase Chain Reaction (PCR) untuk mengamplifikasi gen tersebut kemudian akan diekstraksi untuk memurnikan gen yang dituju. Penelitian ini telah berhasil memurnikan gen 3×Myc PKG-Puro-Poly A. c-MYC (selanjutnya disebut MYC) adalah onkoprotein yang terdiri dari 439 asam amino yang mengandung senyawa DNA terminal-C yang berkarakter baik dan domain transaktivasi terminal-N (TAD). Residu wilayah C-terminal »100 terdiri dari segmen leusin ritsleting-helix-loop-helix (bHLH-LZ) dasar yang mengatur heterodimerisasi antara MYC dan mitranya bHLH-LZ MAX yang memediasi pengikatannya dengan promotor gen.

*Kata Kunci:*
Myc, PCR, Esktraksi

| *Diterima:* | *Disetujui:* | *Online:* |
|---|---|---|
| 19-07-2023 | 11-12-2023 | 31-12-2023 |

## ABSTRACT

*Dysregulation of the oncoprotein c-Myc (Myc) is involved in many types of cancer. Myc is a sequence-specific transcription factor that regulates the transcription of genes involved in the control of cell proliferation and apoptosis through mechanisms that are not well understood. The method of this research is experimental. The experiment result will be described. Some processes will be done by polymerase chain reaction (PCR) to amplify the gene and then it will be extracted to pure the targeted gene. This research has been succesful to purify 3×Myc PKG-Puro-Poly A gene. c-MYC (hereinafter MYC) is an oncoprotein consisting of 439 amino acids contains a well-characterized C-terminal DNA compound and an N-terminal transactivation domain (TAD). The C-terminal region »100 residues comprises a basic leucine zipper-helix-loop-helix (bHLH-LZ) segment that regulates heterodimerization between MYC and its partner bHLH-LZ MAX mediate in their binding to gene promoters.*

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*Keywords:*
*Myc, PCR, Extraction*

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| 2023-07-19 | 2023-12-11 | 2023-12-31 |

## 1. Introduction

Dysregulation of the oncoprotein c-Myc (Myc) is involved in many types of cancer. Myc is a sequence-specific transcription factor that regulates the transcription of genes involved in the control of cell proliferation and apoptosis through mechanisms that are not well understood [1]. The MYC proto-oncogene was originally identified as a cellular gene. Homolog of the viral oncogene (v-myc) of the avian myelocytomatosis retrovirus. MYC gene and its paralogs (MYCN and MYCL) encodes the transcription factors involved in modulation 15% of the global transcriptome from flies to humans. Myc protein has three critical domains: (1) the amino terminal domain (NTD) ports retain the Myc I and II boxes (MBI and MBII) that are present essential for transactivation of Myc target genes; (2) the basics (b) Helix-loop-helix (HLH) DNA binding domain; (3) the carboxy-terminal domain (CTD), which encompasses the Myc-leucine zipper domain. The functionally diverse repertoire of genes controlled by Myc are involved in a wide range of intracellular activity biological processes, including proliferation, differentiation, apoptosis, DNA damage repair, metabolism, and extracellular biological processes events such as angiogenesis and stromal remodeling [2].

There is a high degree of architectural homology motifs in the flanking domains of MYC family members, including the basic region (BR), helix-loop-helix (HLH) and leucine zipper (LZ) at the C-terminus and three extremely conserved regions called MYC boxes 1–3 (MB 1–3) at the N-terminal [3–5]. MYC creates a heterodimer with its co-factor, Max (MYC/Max), via BR, HLH, and LZ motifs requisite for DNA–protein interactions. The chromatin-modifying complex consisting of TIP60, TRRAP, TIP48, and GCN5 recruited by MYC/Max heterodimer propels transcription through binding to the E-box DNA region (CACGTG ) within the regulatory domain of target genes. Accumulation of MYC at the promoter sequences of target genes can also augment the transcriptional activity of genes [3].

Overexpression of MYC occurs in many species human cancer. MYC can be directly activated genetically by chromosomal translocation, genomic amplification, retroviral integration and mutation, and activated by increased gene expression and/or protein stability through activation of other oncogenes, including RAS, SRC, NOTCH or the inactivation of tumor suppressor genes such as APC. Therefore, MYC appears to be a gene product that is frequently induced neoplastic growth [4].

The MYC oncoprotein is a pleiotropic transcription factor that modulates and regulates global gene expression critical cellular processes including proliferation, differentiation, cell cycle, metabolism and apoptosis. Strong evidence supports aberrant MYC expression as a driver of tumor development and maintenance1 and is associated with all of the "characteristic" features of cancer. The role of MYC as a general or specific transcription factor has traditionally been controversial, although more recent studies further support this idea MYC is a general enhancer of highly expressed genes. The MYC oncogene family includes c-MYC, MYCN (N-MYC) and MYCL (L-MYC). The three paralogues have a similar function but show a different expression timing and tissue specificities during development. c-MYC is ubiquitously expressed during tissue development and in a variety of tumors. N-MYC is expressed in neural and early hematopoietic tissue development and is deregulated in various types of cancer types, including neuroblastoma, rhabdomyosarcoma, medulloblastoma, Wilms tumor or retinoblastoma, although it may functionally replace c-MYC in some contexts. L-MYC is particularly expressed in the lungs However, is overexpressed in small cell lung

carcinomas although it is considered to have lower transformation activity. The activity of MYC is normally tightly controlled at the transcriptional and protein levels, but it is estimated that this is the case occurs abnormally in up to 70% of human cancers, many of which are very aggressive (e.g.acute leukemia and high-grade lymphoma) and/or show poor outcomes response to treatment (e.g. small cell lung cancer) and neuroblastoma) [6].

## 2. Metode

The method of this research is experimental. The experiment result will be described. Some processes will be done by polymerase chain reaction (PCR) to amplify the gene and then it will be extracted to pure the targeted gene.

### Material

### Polymerase Chain Reaction (PCR) Components

This kit contains the following components for 200 reactions :

| | |
|---|---|
| KOD -Plus- (1.0 U/μL) * | 200 μL × 1 |
| 10× Buffer for KOD -Plus- | 1.0 mL × 1 |
| 25 mM MgSO4 | 1.0 mL × 1 |
| 2 mM dNTPs | 1.0 mL × 1 |

*The enzyme solution contains anti-KOD DNA polymerase antibodies that neutralize polymerase and 3′→5′ exonuclease activity.

### Standard PCR setup

The following procedure is designed for use with the components included in this kit. Before preparing the mixture, all components except the enzyme solution must be completely thawed.

| Component | Volume | Final Concentration |
|---|---|---|
| 10x Buffer for KOD -Plus- | 5 μL | 1x |
| 2mM dNTPs* | 5 μL | 0.2 mM each |
| 25mM MgSO4 | 2 μL | 1.0 mM |
| 10pmol/μL Primer #1 | 1.5 μL | 0.3 μM |
| 10pmol/μL Primer #2 | 1.5 μL | 0.3 μM |
| Template DNA | X μL | Genomic DNA 10-200 ng/50 μL<br>Plasmid DNA 1-50 ng/50 μL<br>cDNA ≤ 100 ng (RNA equiv.)/50 μL |
| PCR grade water | Y μL | |
| KOD-Plus- (1.0 U/μL) | 1 μL | 1.0 U / 50 μL |
| Total reaction volume | 50 μL | |

### PCR Cycling conditions

The following cycling steps are recommended.

< 2-step cycle >

| | | |
|---|---|---|
| Pre-denaturation: | 94 °C , 2 min. | |
| Denaturation: | 94 °C, 15 sec. | 25-35 cycles |
| Extension: | 68 °C, 1 min./kb | |

< 3-step cycle >

| | |
|---|---|
| Pre-denaturation: | 94 °C , 2 min. |

| | | |
|---|---|---|
| Denaturation: | 94 °C, 15 sec. | 25-35 cycles |
| Annealing: | Tm-[5-10] oC*, 30 sec. | |
| Extension: | 68 °C, 1 min./kb | |

## QIAquick® Gel Extraction Kit

Extract the DNA fragment from the agarose gel using a clean, sharp scraper. Weigh the gel portion into a colorless tube. Add 3 volumes of QG buffer to 1 volume of gel (100 mg gel-100 µL).The maximum amount of gel per spin column is 400 mg. For >2% agarose gel, add 6 volumes of QG buffer. Incubate at 50% for 10 minutes or until the gel portion is completely dissolved. Shake the tube every 2 to 3 minutes to help the gel dissolve. After the gel portion has completely dissolved, check whether the color of the mixture is orange or purple, add 10 µL 3 M sodium acetate, pH 5.0 and mix.The mixture turns yellow.

Add 1 volume of isopropanol to the sample and mix. Place a QIAquick spin column in a provided 2 mL collection tube or vacuum manifold. To bind DNA, add the sample to the QIAquick column and centrifuge for 1 minute or apply vacuum to the collector until all samples have passed through the column.Discard the flow and place the QIAquick column back into the same tube. For sample volumes of 800 > 800 µL, reload and rotate/apply the column.

If the DNA will subsequently be used for sequencing, in vitro transcription, or microinjection, add 500 µL Buffer QG to the QIAquick column and centrifuge for 1 minute or apply vacuum. Discard flow-through and place the QIAquick column back into the same tube. To wash, add 750 µl Buffer PE to QIAquick column and centrifuge for 1 min or apply vacuum. Discard flow-through and place the QIAquick column back into the same tube. Note: If the DNA will be used for salt-sensitive applications (e.g., sequencing, bluntended ligation), let the column stand 2–5 min after addition of Buffer PE. Centrifuge the QIAquick column in the provided 2 ml collection tube for 1 min to remove residual wash buffer.

Place QIAquick column into a clean 1.5 mL microcentrifuge tube. To elute DNA, add 50 µL buffer EBC 10 mM tris-Cl, pH 8.5 or water to the center of the QIAquick membrane, let the column stand for 1 minute, and the centrifuge for 1 minute. After the addition of buffer EB to the QIAquick membrane, increasing the incubation time to up to 4 minutes can increase yield of purified DNA. If the purified DNA is to be analyzed on a gel, add 1 volume of loading dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel as the PCR product.

## 3. Result and Discussion

This research has been succesful to purify 3×Myc PKG-Puro-Poly A gene (Figure 1 and 2). c-MYC (hereinafter MYC) is an oncoprotein consisting of 439 amino acids contains a well-characterized C-terminal DNA compound and an N-terminal transactivation domain (TAD). The C-terminal region »100 residues comprises a basic leucine zipper-helix-loop-helix (bHLH-LZ) segment that regulates heterodimerization between MYC and its partner bHLH-LZ MAX mediate in their binding to gene promoters. On the other side is the TAD consists of residues 1-143 and intrinsically forms one disordered domain regulating MYC biology and is essential for MYC-mediated transcriptional activation. This intrinsic disorder the nature of the MYC structure has been linked to the formation of transcriptional condensates in the superenhancer sites

and, if confirmed, could represent a previously overlooked opportunity to target MYC with specific antineoplastic agents that may be preferentially partitioned in such phase-separated condensates. The next process which can be done is inserting the gene to the plasmid of bacteria by the transformation process [5].

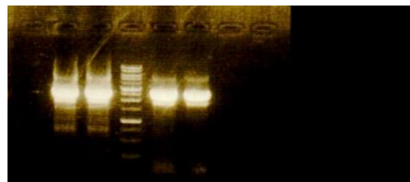

**Figure 1.** 3×Myc PKG-puro-poly A gene before being purified

Some researchs found that c-Myc-deficient MEFs exhibit a reduced ability to proliferate beyond the effects attributable to Cre expression. Furthermore, these defects correlate with the upregulation of the cell cycle inhibitor p27. The potential effects of p27 upregulation are discussed in more detail below. However, we observed that c-Myc-deficient MEFs can proliferate very slowly. The proliferation defect of c-Myc-deficient MEFs is consistent with previous findings that sequential deletion of c-myc genes in RAT1a fibroblasts resulted in prolonged cell doubling time [6]. MYC is the most altered oncogene in human cancer and belongs to a large gene family including MYCN and MYCL. The c-myc proto-oncogene is a superior cell manager that helps allocate resources and control proliferation, growth, differentiation and apoptosis [7] [8].

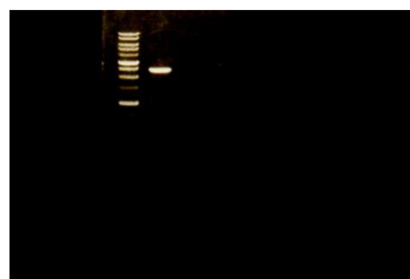

**Figure 2.** 3×Myc PKG-puro-poly A gene after being purified

The heterodimeric transcription factor MYC-MAX has has been documented to bind to consensus DNA sites known as E-boxes (5′-CACGTG-3′) with high affinity and non-consensus sites with lower affinity (Fig. 3). MYC Union to proximal gene promoter sequences facilitate transcription stopped RNA polymerases and catalyzed transcription elongation. It was in this regard that it was proposed that MYC is a general transcription factor that amplifies expression of genes that are already expressed at the basal level, apparently without any specificity ("general reinforcement model). However, the general amplification model does not take into account the ability of MYC to repress genes, such as those activated by the transcription factor MIZ-1. This explanation against this general view of the amplifier hypothesis that MYC targets are largely determined by chromatin accessibility, allowing MYC to bind to target genes and cooperate with other transcription factors to activate or suppress gene expression selectively ("selective amplification"). That is, to what extent MYC stimulates the expression of a gene depends on other transcription factors bound to the gene and/or nearby enhancers [9].

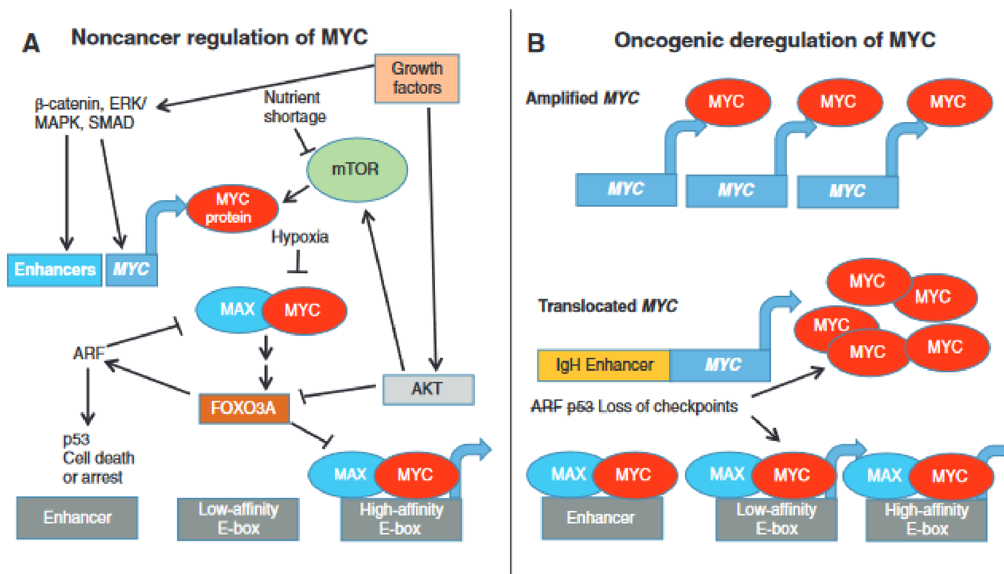


**Figure 3.** Regulation of MYC in cancer and non-cancer cells. A: In noncancerous cells, MYC expression is activated by growth factors activation of amplifiers. The MYC protein, whose translation is enhanced by activated mTOR, dimerizes with MAX to form a heterodimer that becomes activated transcription of genes containing high affinity E-boxes. During nutrient deficiency or hypoxia, MYC translation, protein stability, and MYC/MAX dimerization are inhibited. Overactivation of MYC activates ARF and p53 checkpoints, leading to cell death or arrest, while ARF can inhibit MYC function. Downstream of AKT, FOXO3A proteins counteract MYC activation. B: In cancer cells, constitutive activation of growth factor and mTOR signaling, loss of checkpoints, activation of atypical enhancers, or amplification or translocation of MYC can increase MYC levels to supraphysiological levels independent of growth factors, resulting in binding MYC/MAX leads to decrease. -Affinity binding sites and enhancers in addition to high affinity sites. Loss of ARF or p53 checkpoints enable uncontrolled cell growth.

The binding of Myc and Max to DNA has been analyzed extensively in vitro using recombinant proteins expressed in bacteria, in in vitro transcription/translation systems, and in eukaryotic cells by transfection and transduction with recombinant baculoviruses and vaccinia viruses. Although rapid in vitro transcription/translation systems, they generally yield low levels of Myc/Max proteins that are contaminated with the various proteins in the transcription/translation lysate, including other E-box binding factors.. Eukaryotic cell transfection and viral transduction approaches to overexpression and purification of active Myc:Max dimers are relatively expensive, time-consuming, and require extensive purification steps to remove contaminating proteins and enrich for DNA binding. In fact, Myc proteins, which are overproduced in mammalian CHO cells and in baculovirus-infected insect cells, are phosphorylated and heterogeneous in their apparent molecular mass. Most studies have used bacterial expression systems to produce recombinant Myc and Max proteins with sequence-specific DNA binding activity [10].

As mentioned above, bacterial transformation means the uptake of DNA molecules from the external environment through the cell wall, followed by stable incorporation into the recipient genome or replication as an independent plasmid. Some bacterial species have developed specific mechanisms (competition) for the uptake and

recombination of foreign DNA; This sometimes involves breaking down one strand of the incoming DNA and incorporating the other strand into the chromosome in a type of homologous recombination [11]. Transformation is the process that occurs when a cell absorbs foreign DNA from its environment. In nature, transformations can occur in certain types of bacteria [12]. Cells transformed by constitutive expression of Myc are characterized by loss of expression of numerous genes, suggesting that Myc may also exert a negative function on gene expression [13].

Identification of the 60 kDa protein as a product c-myc coding sequences can be confirmed by analysis in vitro ligated pCM8 expression vector derived from E. coli transcription/translation system. Inclusion of methionine in the in vitro reaction and subsequent gel electrophoresis showed that pCM8 controlled synthesis in this system protein that migrated to and from 60 kDa Protein was synthesized in vivo in E. coli. Several less visible bands that could represent them were also discovered degradation products (the 30 kDa protein is the product) [14]. The sequence of amino acids 290 to 318 of human c-Myc is highly conserved among the c-rnvc genes of different species. However, the N-Myc and L-Myc proteins therein have shorter stretches of discernible homology , which are interrupted by amino acid insertions or deletions (c-Myc residues 295 to 318 show N-Myc homology and c-Myc residues 299 to 314 show L-Myc homology) [15].

## 4. Kesimpulan

This research has been succesful to purify 3×Myc PKG-Puro-Poly A gene. This purified gene can be inserted to bacterial cell by transformation process. The transformation process can use *Escherichia coli* or other bacterial species. The result of transformation is the product of wanted protein such as myc protein.

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