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Original article

In Silico Primer Design for Superoxide Dismutase1 (Sod 1) Gene Amplification in Rattus norvegicus

Zakiya ZT[1]*, Siska Alicia Farma[1]

[1] Departement of Biology, Faculty of Mathematic and Natural Science, Universitas Negeri Padang, Padang, West Sumatera, 25171, Indonesia

*Corresponding author. Tel: +62-83172451227
E-mail: zakiyazerazat@gmail.com

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Abstract

Free radicals are molecules with one or more unpaired electrons, making them unstable and highly reactive. Antioxidants, on the other hand, are compounds that can prevent or inhibit the oxidation of substrates caused by free radicals, even at low concentrations. The body can produce antioxidants enzymatically such as through the action of superoxide dismutase (SOD). To design the primers for our study, we used bioinformatics software available on the National Center of Biotechnology Information (NCBI) and IDT Prime websites. The SOD Rattus norvegicus gene (Z24721.1) was obtained from GenBank NCBI on the NCBI website and converted into the FASTA format. Our results indicated that the forward primer 5'- CAC TTC GAG CAG AAG GCA AG -3' and the reverse primer 5'-TCC CAA TCA CAC CAC AAG CC -3' qualified as good primers, with a ~~primer~~ product (?) length of 236 bp.

Keywords: Free radicals, Antioxidants, Sod gene, Primer design

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INTRODUCTION

Free radicals are highly reactive and unstable molecules that have one or more unpaired electrons (Pangkahila, 2007). They can originate from endogenous sources, which are the. Y-product of normal cell metabolism in the body, or exogenous sources, which come from outside the body, such as air pollution (e.g., cigarette smoke, vehicle smoke, and waste burning smoke). Reactive oxygen species (ROS) is one of the many types of free radicals. It is a product of ormal cell metabolism or cells exposed to ~~other~~ substances that cause inflammation ~~or inflammation~~. ROS includes superoxide, hydroxyl, peroxyl, hydrogen peroxide, singlet oxygen, nitrite oxide, peroxynitrite, hypochlorous acid, and the result of fat oxidation in food. Superoxide is the most commonly produced free radical by the body (Santo *et.al*, 2016).

Superoxide transformed into hydrogen peroxide (H2O2), and in the propagation stage hydrogen is converted into hydroxyl radicals (*OH). The formation of hydroxyl radicals leads to fat peroxidation ~~occurs~~ on the cell membrane, which damaged it. If allowed to continue, this process will result in free radicals with unbalanced antioxidants, leading to oxidative stress (Parwata, 2015).

Antioxidants are low concentrations compounds that can inhibit or prevent the oxidation of substrates in chain reactions caused by free radicals (Halliwell & Whitemann, 2004). Antioxidants can come from exogenous or endogenous sources. Exogenous sources include several types of plants such as vegetables, fresh fruits, various herbs and spices (Irianti *et.al*,. 2017). On the other hand, endogenous sources are produced by the body through enzymatic processes, such as superoxide dismutase (SOD).

Superoxide dismutase is a metalloenzyme that reduces superoxide anion (O_2*) into hydrogen peroxide (H_2O_2), and oxygen (O_2). The highest SOD activity is found in the liver, adrenal glands, kidneys, blood, lymph, pancreas, brain, lungs, stomach, intestines, ovaries, and thymus (Murray *et al.,* 2009). SOD acts as an endogenous antioxidant that reduces oxidant content in the body and prevents oxidative damage by transferring one of its electrons to free radicals that have only a single electron. The high activity of SOD can be observed by the low oxidation products of lipids in each organelle.

Polymerase Chain Reaction (PCR) is the most commonly method used to detect genes in the body. The primer design is the first step in detecting a gene, and the primer can be

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used for DNA amplification using the PCR method. The success of DNA amplification can be affected by using the appropriate primer, as Diss (2003) notes. SOD was identified as erythrocuprein, indophenol oxidase, and tetrazolium oxidase, which contains copper content in blood proteins. The SOD gene is one of the genes that can be used to detect the presence of superoxide dismutase in the body. This gene is located on chromosomes 21, 6 & 4, respectively (21q22.1, 6q25.3 & 4p15.3-p15.1) (Goodsell, 2007). Based on this description, researchers will conduct a primary design of the SOD gene in male white rats (*Rattus norvegicus*) for DNA amplification using the PCR method. Furthermore, the primer is analyzed in silico to obtain a specific primer.

MATERIAL AND METHODS

Time and Place of Research

This research was conducted in January 2023 at the Biological Laboratory, Faculty of Mathematics and Natural Sciences, Padang State University.

Research Tools and Material

The tool used in this study is bioinformatics software on the National Center of Biotechnology Information (NCBI) and IDT Prime sites. The material used is the genome sequence SOD *Rattus norvegicus*. The SOD Rattus norvegicus gene (Z24721.1) was obtained from GenBank NCBI on the https://www.ncbi.nlm.nih.gov/ website and converted into FASTA format (Achiyar *et al.*, 2021).

Primer Design

The primary design was carried out using the FASTA format obtained and processed using Primer Blast software on the NCBI website to obtain the right primary candidate and its specificity in silico using an olygo analyzer on the IDT Prime site by conducting primary sequence tracing.

RESULT AND DISCUSSION

Sod Gene *Rattus norvegicus*

Based on the search results from GenBank NCBI obtained the gene sequence SOD Rattus norvegicus (NM_017050.1) as shown in Figure 1.

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GenBank ▾ Send to:

Rattus norvegicus superoxide dismutase 1 (Sod1), mRNA

NCBI Reference Sequence: NM_017050.1

FASTA Graphics

Go to:

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LOCUS       NM_017050               650 bp    mRNA    linear   ROD 19-DEC-2022
DEFINITION  Rattus norvegicus superoxide dismutase 1 (Sod1), mRNA.
ACCESSION   NM_017050
VERSION     NM_017050.1
KEYWORDS    RefSeq.
SOURCE      Rattus norvegicus (Norway rat)
  ORGANISM  Rattus norvegicus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
            Muroidea; Muridae; Murinae; Rattus.
REFERENCE   1  (bases 1 to 650)
  AUTHORS   Bi D, Shi M, Zheng D, Hu Q, Wang H, Peng L, Lou D, Zhang A and Hu
            Y.
  TITLE     Mechanism underlying the targeted regulation of the SOD1 3'UTR by
            the AUF1/Dicer1/miR-155/SOD1 pathway in sodium arsenite-induced
            liver injury
  JOURNAL   Ecotoxicol Environ Saf 243, 113990 (2022)
   PUBMED   35998476
  REMARK    GeneRIF: Mechanism underlying the targeted regulation of the SOD1
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Figure 1. GenBank gen SOD Rattus norvegicus

The SOD gene *Rattus norvegicus* has a sequence length of 650 bp and is part of the mRNA. Furthermore, the gene sequence is converted to FASTA format so that it can be used in primary design using the Pick Primers tool.

Primer Design

The template is designed so that author affiliations are not repeated each time for multiple authors of the same affiliation. Please keep your affiliations as succinct as possible (for example, do not differentiate among departments of the same organization). This template was designed for two affiliations. Based on the results of the primary blast design from NCBI, 10 pairs of candidates were obtained (Table 1.).

Primers that meet the criteria of a good primer are sod-1 F (5'- CAC TTC GAG CAG AAG GCA AG -3') and sod-1 R (5'- TCC CAA TCA CAC CAC AAG CC -3'). Primer information detail is described in Figure 2. Some things that must be considered in the primer design are the length of the primer, melting temperature (TM), the basic percentage of GC, and Self 3' complementarity (Atifah & Achyar, 2022). In this study, all primers owned have been designed to have lengths ranging from 20-21 bp. The ideal primer has a length of between 18 to 30 oligonucleotides, so that the primer of the ideal length is sufficient to bind to the target gene at

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annealing temperature and obtain a specific sequence (Borah, 2011). To produce a good primer, it is necessary to pay attention to the melting temperature (Tm) of similar forward and reverse primers in the range of 2 to 4°C (Sulistyaningsih, 2007). Primers with too high Tm can result in low PCR products. Whereas Tm that is too low has a tendency.

The stability of a primer affects the pasting of the primer on the template. The stability range of a primer is 1.2 – 2 kcal. If it is too stable then the primer will stick firmly to the template and if it is unstable the primer cannot stick well to the template. In primers that have been designed to have a stability of 1.9 kcal on forward primers and 1.6 kcal on reverse primers, that stability is still within a good primary stability range.

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CACTTCGAGCAGAAGGCAAG	Plus	20	151	170	59.20	55.00	4.00	0.00
Reverse primer	TCCCAATCACACCACAAGCC	Minus	20	547	528	60.54	55.00	2.00	1.00
Product length	397								
Exon junction	165/166 (forward primer) on template NM_017050.1								

Products on intended targets

>NM_017050.1 Rattus norvegicus superoxide dismutase 1 (Sod1), mRNA

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product length = 397
Forward primer  1    CACTTCGAGCAGAAGGCAAG  20
Template        151  ....................  170

Reverse primer  1    TCCCAATCACACCACAAGCC  20
Template        547  ....................  528
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Figure 2. Primer information

Primers that can recognize the sequence of themselves so that they bind to each other and form a structure called a dimer. This can be a problem because primers will tend to stick together, not with the target gene and this can reduce DNA concentrations (Saraswati *et al*., 2019). Things that need to be considered in the selection of primers to minimize the presence of dimer structures in the primer are the number of G-C bonds in the primer and the location of the G-C bonds. A primer with a large number of G-C bonds will encourage the primer to make a dimer rather than bind to the target gene and can also reduce DNA concentration. The location of the G-C bond also needs to be considered because the G-C bond which is located

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close to the end of 3' has a primary tendency to make dimer and will also interfere in the amplification process. Therefore the primer chosen is the primer with the least number of G-C bonds to avoid the presence of a dimer structure.

Table 1. The candidates primer of SOD1 gene *Rattus norvegicus.*

Primer	Sequence	Length	Tm	GC %	Self complementary
Forward	CACTTCGAGCAGAAGGCAAG	20	59,20	55,00	0,00
Reverse	TCCCAATCACACCACAAGCC	20	60,54	55,00	1,00
Forward	ACTTCGAGCAGAAGGCAAGC	20	60,95	55,00	2,00
Reverse	GGGCAATCCCAATCACACCA	20	60,61	55,00	0,00
Forward	GGCCGTACTATGGTGGTCC	19	59,56	63,16	3,00
Reverse	CCAATCACACCACAAGCCAAG	21	60,00	52,38	0,00
Forward	GCGGATGAAGAGAGGCATGTT	21	60,75	52,38	0,00
Reverse	TTCCACCTTTGCCCAAGTCAT	21	60,13	47,62	2,00
Forward	CGGATGAAGAGAGGCATGTTG	21	59,06	52,38	1,00
Reverse	TTGGGCAATCCCAATCACAC	20	58,73	50,00	0,00
Forward	CTTCGAGCAGAAGGCAAGCG	20	61,96	60,00	2,00
Reverse	TTTCCACCTTTGCCCAAGTCA	21	60,34	47,62	1,00

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Forward	ATGGGGACAATACAC AAGGCTG	22	60,62	50,00	1,00
Reverse	AATCCCAATCACACC ACAAGC	21	59,10	47,62	2,00
Forward	TTGGCCGTACTATGG TGGTCC	21	61,85	57,14	3,00
Reverse	TTGGGCAATCCCAAT CACACC	21	61,17	52,38	0,00
Forward	GGACAATACACAAG GCTGTACC	22	58,99	50,00	2,00
Reverse	TCACACCACAAGCCA AGCG	19	61,19	57.89	2,00
Forward	TTCACTTCGAGCAGA AGGCAAG	22	61,12	50,00	0,00
Reverse	TTCACTTCGAGCAGA AGGCAAG	21	58,75	47,62	0,00

Conclusion

The primer design created in silico for the sod-1 gene sequence of *Rattus norvegicus* produces a pair of sequences, with a forward primer of 5'- CAC TTC GAG CAG AAG GCA AG -3' and reverse primer 5'-TCC CAA TCA CAC CAC AAG CC -3'. These primers meet the criteria of ideal primers and are ready for further in vitro PCR amplification processes.

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