

DESAIN PRIMER UNTUK ANALISIS SEKUEN GEN *MC1R*, RESEPTOR GEN PENENTU PIGMENTASI RAMBUT DAN KULIT

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INTISARI

Gen *MC1R* mengkode protein yaitu *melanocortin 1 receptor* yang berperan sebagai reseptor pada proses melanogenesis. Mutasi pada gen *MC1R* dapat meningkatkan jumlah pigmen pheomelanin yang menyebabkan seseorang memiliki rambut merah, *fair skin*, dan *freckles*. Gen *MC1R* dapat dideteksi dengan menggunakan primer spesifik melalui *Polymerase Chain Reaction* (PCR) pada proses amplifikasi. Primer spesifik untuk deteksi gen *MC1R* tersebut dapat didesain secara *in silico* menggunakan *software*. Berdasarkan hal tersebut, penelitian ini bertujuan untuk mendesain primer spesifik secara *in silico* yang dapat digunakan untuk mengamplifikasi gen *MC1R*. Pada penelitian ini dilakukan beberapa tahap yaitu melakukan pendataan data sekuen gen *MC1R* dari *database GenBank* (*accession number* NC_000016.10), mendeteksi *Single Nucleotide Polymorphism* (SNP), mendesain primer menggunakan *software* Primer3Plus, menganalisis struktur sekunder (*secondary structure*) pada kandidat primer menggunakan program NetPrimer PREMIER Biosoft, dan selanjutnya diuji secara *in vitro* menggunakan metode *Polymerase Chain Reaction* (PCR). *Single Nucleotide Polymorphism* (SNP) yang dipetakan pada gen *MC1R* terletak pada bagian CDS dengan panjang fragmen 954 bp dan tersusun atas 317 asam amino. Bagian CDS menjadi target untuk desain primer menggunakan *software* Primer3Plus. Adapun protein *MC1R* memiliki motif asam amino yaitu asam aspartat, arginin, dan tirosin (DRY) yang berperan dalam aktivasi reseptor dan terletak pada pembatas domain transmembran 3 (TM3) dan *intracellular loop 2* (IL2). Pada penelitian ini didapatkan tiga pasangan primer *forward* dan *reverse* terbaik yaitu *MC1R1* (*forward* *MC1R1_F*: GAGGTGTCCATC TCTGACG dan *reverse* *MC1R1_R*: ATGAGCACCAGCATAGCC); *MC1R2* (*forward* *MC1R2_F*: CATCTCTGACGGGCTCTT dan *reverse* *MC1R2_R*: ATGAGCACCAGCATAGCC); dan *MC1R3* (*forward* *MC1R3_F*: ATCTCTGACG GGCTCTTCC dan *reverse* *MC1R3_R*: GCCATGAGCACCAGCATA) berdasarkan kriteria panjang primer, panjang fragmen ampikon, *GC content*, *melting temperature* (*T_m*), struktur sekunder primer (*self-dimer*, *cross-dimer*, dan *hairpin*), *repeats*, dan *runs*.

Kata kunci: Gen *MC1R*, desain primer, Primer3Plus, NetPrimer, PCR

PRIMER DESIGN FOR SEQUENCE ANALYSIS OF *MC1R* GENE, RECEPTOR FOR HAIR AND SKIN PIGMENTATION GENES

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ABSTRACT

The *MC1R* gene encodes a protein, melanocortin 1 receptor, that acts as a receptor in the melanogenesis process. Mutations in the *MC1R* gene can increase in the amount of pheomelanin pigment that causes a person to have red hair, fair skin, and freckles. The *MC1R* gene can be detected using specific primers through Polymerase Chain Reaction (PCR) in the amplification process. Specific primers for the detection of the *MC1R* gene can be designed through *in silico* using software. Therefore, this study aimed to design specific primers through *in silico* that can be used to amplify the *MC1R* gene. In this study, several stages were conducted, firstly was to determine the *MC1R* gene sequence data from GenBank database (accession number NC_000016.10), secondly was to detect single nucleotide polymorphism (SNP) data, thirdly was to design specific primers using Primer3Plus software and analyze secondary structure in primer candidates using NetPrimer PREMIER Biosoft program, and then tested through *in vitro* using a Polymerase Chain Reaction (PCR) method. In this study, Single Nucleotide Polymorphism (SNP) mapped on the *MC1R* gene was located on a CDS region with a fragment length of 954 bp and consist of 317 amino acid. The CDS region was determined as the primer design target using Primer3Plus software. The MC1R protein has amino acid motifs, that is aspartic acid, arginine, and tyrosine (DRY) which play a role in receptor activation and is located at the boundary of transmembrane domain 3 (TM3) and intracellular loop 2 (IL2). The result revealed three best forward and reverse primer pairs were MC1R1 (forward MC1R1_F: GAGGTGTCCATCTCTGACG and reverse MC1R1_R: ATGAGCACCAGCATAGCC); MC1R2 (forward MC1R2_F: CATCTCTGACGG GCTCTT and reverse MC1R2_R: ATGAGCACCAGCATAGCC); and MC1R3 (forward MC1R3_F: ATCTCTGACGGGCTCTTCC and reverse MC1R3_R: GCCATGAGCACCAGCATA) based on primer length criteria, amplicon fragment length, GC content, melting temperature (T_m), primary secondary structure (self-dimer, cross-dimer, and hairpin), repeats, and runs.

Keywords: *MC1R*, primer design, Primer3Plus, NetPrimer, PCR