

EXPRESSION OF EXON 5 GENE ENCODING *Sarcoptes scabiei* varian *hominis* TROPOMYOSIN PROTEIN

ABSTRACT

Background: Scabies is a skin disease caused by infestation of the mite *Sarcoptes scabiei*, and it belongs to the neglected tropical disease with high burden of disease in poor and developing countries. Indonesia is the country with the highest burden of scabies. Various diagnostic criterias lead to high differences in the examination results. Currently there are no uniform diagnostic procedure for diagnosing scabies.

Purpose: This study aimed to express the exon 5 gene encoding tropomyosin protein of the *Sarcoptes scabiei* varian *hominis*.

Methods: This was a quasi-experimental study. The 550 bp fragment of the *Sarcoptes scabiei* 5th exon tropomyosin gene was cloned using pLATE-51, a plasmid with ligation independent cloning system. Overexpression was carried out using competent bacteria *Eschericia coli* BL21(DE3) at 37°C and 20°C. Induction was carried out by adding IPTG with a concentration of 0.1 mM; 0.5mM; 0.75mM, 1mM; and 2mM. Analysis of the overexpression results was carried out by SDS-PAGE electrophoresis. Confirmation of the presence of recombinant protein was performed by immunodot-blotting and western blotting, using *6x His Tag Monoclonal Antibody* (Invitrogen) as the primary antibody and *Goat-anti mouse IgG2b* (Invitrogen) as the secondary antibody.

Results: *S. scabiei* exon 5 tropomyosin gene amplification using specific primers produced 550 bp amplicon. Amplicons or PCR products were then cloned into the pLATE-51 vector, a plasmid using the ligation independent cloning method. Analysis of the results of overexpression with SDS-PAGE electrophoresis produced many bands. The resulting protein is predicted to have a molecular weight of around 20 kDa. Immunodot-blotting results show that there is a point which is the recognition of recombinant protein by the antibody used. The western-blotting profile shows a band about 20 kDa in size that is recognized by the antibody.

Conclusion: The exon 5 tropomyosin gene of *S. scabiei* could be amplified with specific primers and cloned on the plasmid pLATE-51. The resulting recombinant protein is about 20 kDa in size.

Keywords: Scabies, *Sarcoptes scabiei*, recombinant protein, tropomyosin, cloning, ligation independent cloning

EKSPRESI EKSON 5 GEN PENYANDI PROTEIN TROPOMYOSIN DARI *Sarcoptes scabiei* varian *hominis*

INTISARI

Latar belakang: Skabies adalah penyakit kulit yang disebabkan infestasi tungau *Sarcoptes scabiei*, dan termasuk ke dalam kelompok *neglected tropical disease* dengan beban penyakit yang cukup tinggi pada negara berkembang. Indonesia menjadi negara dengan beban penyakit skabies tertinggi. Kriteria diagnosis yang beragam menyebabkan tingginya perbedaan dalam hasil pemeriksaan. Hingga saat ini, belum ada prosedur diagnostik yang seragam untuk mendiagnosis skabies.

Tujuan: Penelitian ini bertujuan untuk mengekspresikan ekson 5 gen penyandi protein tropomyosin *Sarcoptes scabiei* varian *hominis*.

Metode: Penelitian ini menggunakan desain eksperimental semu. Ekson 5 gen penyandi protein tropomyosin *Sarcoptes scabiei* yang berukuran 550 bp dikloning menggunakan pLATE-51, sebuah vektor plasmid *ligation independent cloning*. Overekspresi gen penyandi tropomyosin dilakukan pada bakteri kompeten *Escherichia coli* BL21(DE3) yang di tumbuhkan pada suhu 37°C dan 20°C. Induksi dilakukan dengan penambahan IPTG dengan konsentrasi 0,1 mM; 0,5 mM; 0,75 mM, 1 mM; dan 2 mM. Analisis hasil overekspresi dilakukan dengan elektroforesis SDS-PAGE. Konfirmasi keberadaan protein rekombinan dilakukan dengan *immunodot-blotting* dan *western blotting*, menggunakan antibodi *6x His Tag Monoclonal Antibody* (Invitrogen) sebagai antibodi primer dan *Goat-anti mouse IgG2b* (Invitrogen) sebagai antibodi sekunder.

Hasil: Amplifikasi ekson 5 gen tropomyosin *S. scabiei* menggunakan primer spesifik menghasilkan ampikon berukuran 550 bp. Ampikon atau produk PCR kemudian dikloning pada vektor pLATE-51, sebuah plasmid dengan metode *ligation independent cloning*. Analisis hasil overekspresi dengan elektroforesis SDS-PAGE menghasilkan banyak pita. Protein yang dihasilkan diprediksi memiliki berat molekul sekitar 20 kDa. Hasil *immunodot-blotting* menunjukkan adanya titik yang merupakan pengenalan protein rekombinan oleh antibodi yang digunakan. Profil *western-blotting* menunjukkan pita dengan ukuran sekitar 20 kDa yang dikenali oleh antibodi.

Kesimpulan: Ekson 5 gen penyandi protein tropomyosin *S. scabiei* dapat diamplifikasi dengan primer spesifik dan diklonasi pada plasmid pLATE-51. Protein rekombinan yang dihasilkan berukuran sekitar 20 kDa.

Kata kunci: Skabies, *Sarcoptes scabiei*, protein rekombinan, tropomyosin,
kloning, *ligation independent cloning*