

# Analisis Genom HPV 16 Galur UI66 dari Indonesia dan Pengklonaan Fragmen E6/E7 sebagai Kontrol Positif Deteksi HPV 16 = Genome analysis of HPV 16 UI 66 isolate from Indonesia and cloning of E6/E7 fragment as positive control for HPV 16 detection

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## Abstrak

[HPV 16 merupakan genotipe yang paling banyak terdeteksi pada kanker serviks. Telah diketahui adanya berbagai varian HPV 16 yang di kategorikan berdasarkan distribusi geografisnya. Broer et al melaporkan varian HPV16 di Indonesia berdasarkan analisis gen E6, E7 dan L1. Belum ada jurnal yang melaporkan analisis gen-gen lain dari HPV 16 isolat Indonesia. Metode deteksi dan genotyping merupakan metode utama deteksi infeksi dan uji keberhasilan vaksin HPV. Saat ini metode deteksi dan genotyping HPV berbasis gen E6/E7 sedang dikembangkan karena gen E6 dan E7 merupakan gen yang paling lestari pada pasien kanker serviks. Standar dan kontrol untuk akurasi deteksi dan genotyping sangat diperlukan. WHO telah menyediakan standar internasional berupa plasmid rekombinan berisi genom lengkap HPV 16 dan 18 dari materi genetik HPV luar negeri. Oleh karena itu pada penelitian ini dilakukan pengklonaan fragmen E6/E7 dari HPV 16 dari Indonesia yang dapat digunakan sebagai kontrol positif deteksi HPV 16. Fragmen-fragmen dari genom HPV 16 galur UI66 diamplifikasi dengan PCR dan disekuensing kemudian dianalisis variasi genetik gen E6, E7, E1, E2, E4, E5, L2, parsial L1 dan LCR dan dibandingkan dengan HPV 16 referensi. Dilakukan pengklonaan fragmen 1 yang mengandung gen E6/E7 dan diujikan sebagai kontrol positif deteksi HPV 16 dengan menggunakan PCR dan real time PCR. Hasil penelitian menunjukkan tingkat similaritas nukleotida dan asam amino masing-masing gen galur UI66 bervariasi dibanding dengan HPV 16 referensi dengan similaritas tertinggi berturut-turut adalah parsial gen L1 dan protein E7. Kekerabatan masing-masing gen dari galur UI66 sangat bervariasi. Telah diperoleh 1 klon yang membawa plasmid rekombinan pUI66F1-E6/E7 yang dapat digunakan sebagai kontrol positif deteksi HPV 16 dengan target gen E6 dan E7.; HPV 16 is the most commonly detected genotype of HPV in cervical cancer. HPV 16 variants have been categorized based on their geographical distribution. Broer et al. have discovered HPV16 variants in Indonesia based on analysis of E6, E7 and L1. There has been no reports of other genes of HPV-16 Indonesian isolates. Methods for detection and genotyping of HPV DNA are the primary tools used to measure HPV infection and vaccine impact. Today, detection and genotyping HPV based on E6/E7 genes are being developed as E6 and E7 genes are the most conserved genes in cervical cancer patients. Standards and controls for the detection and genotyping accuracy are needed. WHO provides international

standards in the form of a recombinant plasmid that contains the complete genome of HPV 16 and 18 from HPV genetic material abroad. Therefore in this research, cloning of fragments of HPV 16 E6/E7 Indonesian isolate have been done and used as a positive control for detection of HPV 16. The genome fragments of the HPV 16 UI66 isolates were amplified by PCR and sequenced then analyzed genetic variations of E6, E7, E1, E2, E4, E5, L2, partial L1 genes and LCR compared with HPV 16 reference. Cloning of fragment 1 contain E6/E7 genes have been done and tested as positive control of HPV 16 detection by real time PCR. The result showed the nucleotide and amino acid similarity of UI66 isolates varied with the highest value were partial L1 gene and E7 protein respectively. Phylogeny of each gene from isolate UI66 is variable. One clone was obtained carrying the recombinant plasmid pUI66F1-E6/E7 that can be used as a positive control for detection of HPV 16 which E6 and E7 genes as targets., HPV 16 is the most commonly detected genotype of HPV in cervical cancer. HPV 16 variants have been categorized based on their geographical distribution. Broer et al. have discovered HPV16 variants in Indonesia based on analysis of E6, E7 and L1. There has been no reports of other genes of HPV-16 Indonesian isolates. Methods for detection and genotyping of HPV DNA are the primary tools used to measure HPV infection and vaccine impact. Today, detection and genotyping HPV based on E6/E7 genes are being developed as E6 and E7 genes are the most conserved genes in cervical cancer patients. Standards and controls for the detection and genotyping accuracy are needed. WHO provides international standards in the form of a recombinant plasmid that contains the complete genome of HPV 16 and 18 from HPV genetic material abroad. Therefore in this research, cloning of fragments of HPV 16 E6/E7 Indonesian isolate have been done and used as a positive control for detection of HPV 16. The genome fragments of the HPV 16 UI66 isolates were amplified by PCR and sequenced then analyzed genetic variations of E6, E7, E1, E2, E4, E5, L2, partial L1 genes and LCR compared with HPV 16 reference. Cloning of fragment 1 contain E6/E7 genes have been done and tested as positive control of HPV 16 detection by real time PCR. The result showed the nucleotide and amino acid similarity of UI66 isolates varied with the highest value were partial L1 gene and E7 protein respectively. Phylogeny of each gene from isolate UI66 is variable. One clone was obtained carrying the recombinant plasmid pUI66F1-E6/E7 that can be used as a positive control for detection of HPV 16 which E6 and E7 genes as targets.]