



## HPV16 L1在整合型重组毕赤酵母中的表达及病毒样颗粒的纯化

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Expression and purification of HPV16 L1 virus-like particles in integrated recombinant *Pichia*

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摘要

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**摘要** 目的 构建可稳定表达HPV16 L1的整合型重组毕赤酵母, 并纯化自主组装成的HPV16 L1病毒样颗粒(VLPs)。方法 根据酵母密码子偏爱性优化HPV16 L1基因并克隆到pPIC3.5K表达载体, 构建pPIC3.5K/HPV16 L1重组质粒; 重组质粒经*Bgl* II酶切线性化后, 电转化至GS115菌株中, 筛选HPV16 L1重组毕赤酵母。阳性整合菌株甲醇诱导后, 以HPV16 L1单克隆抗体检测目的蛋白表达; 采用肝素亲和层析法纯化HPV16 L1VLPs并进行透射电镜观察。结果 PCR、酶切和测序分析表明成功构建了pPIC3.5K/HPV16 L1重组质粒。成功构建的HPV16 L1重组毕赤酵母甲醇诱导后, Western blot证实重组酵母菌裂解产物存在HPV16 L1目的蛋白。肝素亲和纯化后, 透射电镜观察到了直径大约55 nm的VLPs, 其形态与HPV16天然病毒颗粒相似。结论 利用整合型重组毕赤酵母表达系统成功表达了HPV16L1蛋白, 并用肝素亲和纯化可快速获得结构完整的HPV16 L1VLPs, 为HPV16预防性疫苗的研制奠定基础。

**关键词:** 人乳头瘤病毒 主要衣壳蛋白L1 毕赤酵母表达系统 病毒样颗粒

**Abstract:** In order to produce HPV16 L1 protein and self-assemble virus-like particles (VLPs), optimized HPV16 L1 gene according to the

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yeast cell codon preference was synthesized and cloned into pPIC3.5K yeast expression vector. After linearized with *Bgl* II restriction enzyme, pPIC3.5K/HPV16 L1 recombinant plasmid was electroporated into GS115 strain to construct HPV16 L1-recombinant *Pichia pastoris*. Identified recombinant strains were then induced by methanol. The expression of HPV16 L1 was identified by monoclonal antibodies, and self-assembled VLPs were purified by heparin and observed under transmission electron microscope. Data of PCR detection, restriction enzyme digestion and sequencing analysis showed that pPIC3.5K/HPV16 L1 recombinant plasmid was successfully constructed. Western blot verified the expression of HPV16 L1 in the lysate of HPV16 L1 recombinant *Pichia pastoris*. After purified by Heparin, VLPs with diameter of about 55 nm were observed in the transmission electron microscopy. Their shapes were similar to natural HPV16 virus particles. In conclusion, we successfully expressed HPV16 L1 protein in integrated recombinant *Pichia pastoris* system and quickly obtained intact conformation of VLPs by using heparin affinity chromatography. This study will facilitate the development of HPV16 prophylactic vaccine.

Keywords: [human papillomavirus \(HPV\)](#) [major capsid protein L1](#) [Pichia pastoris expression system](#) [virus-like particles \(VLPs\)](#)

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