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ENU诱导带LacZ靶基因的λgt11 DNA突变分子机理的初步研究①
Mutation Study of λgt11 DNA with LacZ Induced by ENU

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摘要　采用ENU(乙基亚硝基脲)作用于裸露的 λgt11 DNA，经体外重包装，转染宿主菌　E. coli Y1090，在含底物X-gal，诱导剂IPTG的选择性培养基上铺皿，发现被处理的 λgt11 DNA除了使噬菌体存活率下降外，还出现了靶基因“LacZ”较高频率的突变。其中以二甲基亚砜(DMSO)为溶剂，当存活率分别为3.5×10-3、1.6×10-3和5.5×10-4 时，相应的突变率依次为1.1×10-3、3.2×10-3和5.2×10-3，DMSO溶剂对照突变率则<5.0×10-5 。对ENU诱导的5个阳性突变体进行了扩增，以PCR产物为模板，采用正向引导，对阳性突变体靶基因LacZ进行了部分测序，在被测序的260bp范围内，发现了9个位点的碱基突变。碱基突变的类型有颠换(67%)、转换(11%)和移码突变(22%)。颠换主要以A→T、G→C为主。似乎胞嘧啶(C)更易发生突变(占43%)。

Abstract　To construct molecular mutation detective system of λDNA with LacZ, naked λgt11 DNA was treated with mutagen ENU (Ethylnitro sourea). The ENU-damaged DNA was added to Lambda packaging extracts and the resulting phage were grown in host　E.coli　Y1090 on a selective plate containing substract X-gal and inducer IPTG. Under these conditions, the results showed that the higher the viability ratio was, the lower the frequency of clear-plaque mutants occured. In our study, when survival ratios of the host cell survival ratio were 3.5×10-3、1.6×10-3　and 5.5×10-4 respectively, the mutation ratio were 1.1×10-3、3.2×10-3　and 5.2×10-3　accordingly, and the mutation ratio by DMSO (negative control) was below 5.0×10-5. 260 bases from ENU—induced LacZ gene were subjected to DNA sequence analysis. There were several mutation sites: transversion (6, 67%), transition(1, 11%), frame shift(2, 22%)(both were insert mutation). Transversions mainly consisted of A→T, G→C. Among the four bases, cytosine seemed to be more sensitive to ENU (43%).

关键词　LacZ基因　突变　体外重包装　诱变剂　DNA测序 Key words　KLacZ　Mutation　in vitro　repackage　Mutagen sequencing　DNA squence

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Abstract

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