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酵母脯氨酸合成酶基一因（Pro2）的分离及Leu' Pro十表型共转导

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摘要 酵母7209一1 A (a) DNA，用pHC79为载体，以BamHI, PstI和Hind III 酶切，经体外重组和包装后，转导至大肠杆菌HB101和SC294中。在我们的实验条件下（对酵母DNA适度酶解，F/V值为15，连接时DNA总浓度为200-250微克 / 微升以及BHB 2688 / BHB 2690比值为5)，重组建库效率达到106 CFU f微克载体DNA，重组子双抗值降低到10％或5％以下。

在大肠杆菌HB 101和SC 294的106个重组体克隆中，观察到酵母基因对IcuB 6及proA2（在HB101中），leu6, metB1, argG, 11is1（在SC294中）等营养缺陷型的互补（校正）效应。由于互补频率(10-3-10-4,）比上述基因突变自发回复率高2-3个数量级，因此可以说，酵母的上述基因在大肠杆菌中获得了功能表达。对pyeleu5和pYcleu7单克隆DNA.的再次包装转导和再次互补测试表明，我们已使酵母leu+的互补效率提高109佑。本文还报道了在HB101中的酵母基因文库Leu+ Pro+的表型共转导现象，频率为30％以上。

关键词

分类号

Cloning of Yeast Gene (Pro2）Coding for Proline Synthesizing Enzyme（Glutamate Phosphate Reductase）and Leu＋Pro' Phenotypic Cotransduction

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Abstract

<P><FONT face=Verdana> Six gene libraries of Saccharomyces cerevistae 7209-1A（a) were constructed efficiently ith cosmid pHC79 DNA ligated at their BamHI, PstI, and HindIII sites,using HB101 and SC294 as recipient strains.
 Several factors affected the construction of the libraries. With proper extent of he restriction enzyme digestio·n of yeast DNA, F/V ratio for ligation at 15/1 DNA concentration for ligation at 200-250 Vg/ml and BHB2688/BHB2690 for in vitro packaging t 5/1, we got rather high efficiency of library construction （106 CFU/pg vector NA）and low Apr Ter％ （down to less than 10％ or even 5％），
 We found the complementation (supression) effect on the auxotrophs of leuB, proA in HB101），leu6, inetB1, hisl, and aro G6 (in SC294, a gift from Dr. G. Hobom）. The requencies of complementation of these six genes are between 10一a-10'4, while the pontaneous reversion rates in leu, pro, his, met, and arc, mutants are lower than 10-6 Aa rsult, we conclude that genes（.Lent, Pro, Met, His, and Arg）on the inserted frag.-ment of yeast DNA in the hybrid plasmid can be functionally expressed in E doli.
 We also found high frequency（＞30％）of cotransducti}on of Pro+ with Leu+ phenotype n yeast gene library in HB101. We have concentr一ated yeast Leu2 gene or supressor or more than 7_04 time; after repackaging and retesting for complementation w.it't Yeleu5 and pYeleu7 DNA.</FONT></P>

Key words

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