遗传学报 1990 17 (1): 38-45 ISSN: 0379-4172 CN: 11-5450/R

cosmid克隆筛选的体内同源重组法——一个含有小鼠t复合体连锁DNA顺序的cosmid克隆的分离

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收稿日期 修回日期 网络版发布日期 接受日期

摘要 一个从cosmid分子克隆库中筛选特别基因顺序的遗传学方法——体内同源重组(invivo homo logous recombination)法。即使探针DNA与分子克隆库中带有与探针同源顺序的克隆发生体 内重组，然后以遗传学方法进行筛选。cosmid分子克隆库构建在rec-宿主细胞内，经体内包 装(in vivo Packaging)成λ噬菌体颗料，把该噬菌体颗粒，把该噬菌体颗粒转入带有探针D NA的rec+细胞内，探针是已被克隆在与cosmid载体没有同源顺序的质粒(如PUC8或PUC9)内的 。经过一段时间(1-3小时)，待重组发生后，把cosmid进行体内包装。此时探针DNA连同质粒 已整合入cosmid基因组内，因此它带有原为两个载体所分别带有的双重抗性——Amp r(氨苄 青霉素，PUC8或PCU9)和Kan r(卡那霉素，cosmid)。这种双重抗性菌落可在含有这2种抗菌 素的培养平皿上选出，该重组cosmid借且于λ切除酶的作用将已被整合的探针质粒重新切除 ，再经体内包装后，该cosmid被还原并纯化，然后可用一含有Xgal的培皿识别和选出。本文 用此法以有关DNA探针从cosmid分子克隆库中分离得到含有与小鼠t复合体连锁的基因组顺序 的克隆，并对该克隆作了物理图谱分析。

关键词 重组DNA,体内同源重组,体内包装,cosmid分子克隆库,基因筛选

分类号

The Selective Isolation of Cosmid Clones by Homologous Recombination in Escheric hia coli— A Cosmid Clone Containing t Complex Linkage DNA Sequence of Mouse Wa s Isolated

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Abstract

A procedure for the selective isolation of specific cosmid clones by homologous recombination between cosmid clones of genomic library and a probe DNA sequence cloned in a plasmid in vivo has been developed.The cosmid library was constructe d in a rec- host cell strain and packaged into phage particles in vivo.The rec+ host cells containing a DNA sequence used as selection probe cloned in the pUC p lasmid were infected by packaged cosmid phage particles.There is no homology bet ween cosmid and the plasmid vectors.After a period of 1-3hr.for the recombinatio n to take place,the probe plasmids were integrated into cosmid,in which the DNA sequence are homologous with the probe,by homologous recombination.The cosmids a re then packaged in vivo and transferred into a rec- cell strain.The specific cos mid clones were selected by double antibiotic resistance carried by both vetors. The probe plasmid can be excised by λ excision enzyme by using superinfection w ith red+ phage.After packaging in vivo,these cosmid revertants can be identified on Xgal plate. A cosmid clone containing the t complex linkage DNA sequence of mouse was se lected by using the procedure above with a probe derived from microdissected met aphase chromosome.

Key words Recombinant DNA Cosmid library Cosmid selection Homologous recombination Packagi ng in vivo

DOI:

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