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正常人体淋巴细胞cDNA文库的构建 Construction of Normal Human Lymphocyte cDNA Library

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摘要　应用GIBCOBRL建库试剂盒建立了正常人体淋巴细胞cDNA文库。取新鲜的正常人外周血，分离出淋巴细胞，进行体外培养，提取总RNA，纯化mRNA，并将其反转录成cDNA，与SalI和NotI接头连接后插入λZipLox载体，体外包装后转染到Y1090宿主菌中，进行滴度测试及文库扩增。构建的正常人淋巴细胞cDNA文库含2-6×106重组子，克隆效率为5×1012重组子/g cDNA，插入片段长度约为1~5kb。扩增后的文库浓度为3×107重组子/μl，将文库稀释到10-6时所产生的噬菌斑密度最为适宜。试验结果表明，该库符合标准，所构建的正常人淋巴细胞cDNA文库为进一步筛选目的基因、制作基因芯片等提供了有效的工具。

Abstract:A lymphocyte cDNA library of normal human was constructed in order to obtain specific gene and prepare lymphocyte gene chips to detect the relative genes between psychiatric diseases and immunity. The lymphocyte was abstracted from fresh normal human blood and cultured in vitro. Total RNA of lymphocyte was extracted from the cultured cells and then mRNA was extracted further. Moreover, single-strand cDNA and double-strand cDNA were synthesized in turn. The double-strand cDNAs were ligated to SalI and NotI adaptor, which were later ligated to arms of λ ZipLox. Ligated-cDNAs were packed in vitro, and then infected E. coli Y1090. Titering the phage and amplifying the library. The lymphocyte cDNA library consisted of 2-6×106 recombinants with the length of 1~5kb and the cloning efficiency was 5×1012 recombinants/g cDNA. The amplified library was 3×107recombinants/μl in concentration and the number of bacteriophage plagues was the most suitable in density after it was diluted to 10-6 in concentration. The constructed cDNA library of normal human lymphocyte would be helpful to further detecting target genes and preparing gene chips etc.

关键词　cDNA文库　构建　外周血　淋巴细胞 Key words　cDNA library　construction　circulation blood lymphocyte

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Abstract

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