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人源性抗核抗体Fab片段的筛选及鉴定 点此下载全文

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摘要：

目的：制备人源性抗核抗体Fab片段。方法：通过4轮淘筛，富集已构建的人源性抗核抗体Fab片段噬菌体抗体库，间接ELISA法鉴定4轮淘筛后抗核抗体Fab片段噬菌体抗体；提取阳性克隆的噬菌粒DNA，切除gIII基因片段，自连接后转化大肠杆菌XL1-Blue，以IPTG诱导表达可溶性人源性抗核抗体Fab片段；应用间接ELISA法及荧光免疫法对表达上清进行鉴定。结果：第4轮洗脱的噬菌体滴度较第1轮增加200余倍，从抗核抗体Fab片段噬菌体库中筛选出2株阳性克隆，切除gIII基因后自连的噬菌粒DNA经Xho I 单酶切证实连接成功。间接ELISA法检测结果显示：制备的可溶性人源性抗核抗体Fab片段均呈现抗dsDNA阳性，具有抗原特异性；免疫荧光法结果显示：Hep2细胞和猴肝脏组织细胞核显示均质型荧光，绿蝇短膜虫的动基体显示均质型荧光。结论：成功制备具有抗原特异性的可溶性、人源性抗核抗体Fab片段，为高亲和力抗核抗体Fab片段的制备奠定了基础。

关键词：抗核抗体 单克隆抗体 噬菌体展示肽库 筛选

Panning and identification of humanized antinuclear antibody Fab fragment Download Fulltext

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Abstract:

Objective: To prepare humanized antinuclear antibody Fab fragment. Methods: The reconstructed humanized antinuclear antibody (ANA) Fab phage display library was enriched by 4 rounds of panning and was identified by indirect ELISA method. Phasmid DNA isolated from positive clones was deprived of gIII gene. After self-ligation the recombinant plasmid was used to transform E. coli. XL1-Blue, then XL1-Blue was induced by IPTG to product soluble human antinuclear antibody Fab fragment. Finally, soluble human antinuclear antibody Fab in the supernatant was identified by indirect ELISA method and immunofluorescence. Results: The eluted phages were enriched by more than 200 folds after 4 rounds of panning. Two positive clones were isolated from the ANA Fab library. Electrophoresis after Xho I digestion proved that the self-ligation was successful after deletion of gIII gene. The results of indirect ELISA indicated that the 2 positive clones of Fab had specific anti-dsDNA activity. Indirect immunofluorescence showed homogeneous fluorescence within nuclei of Hep2 and monkey hepatic cells and in the Crithidia kinetoplast. Conclusion: We have successfully prepared soluble, specific human antinuclear antibody Fab fragment, which paves a way for preparation of high affinity antinuclear antibody Fab fragment.

Keywords: antinuclear antibodies monoclonal antibody phage display peptide library panning

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