

论文

Lipocalin文库的构建及与克百威有结合活性的小分子筛选

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摘要:

构建大菜粉蝶Lipocalin蛋白家族中的BBP蛋白突变文库, 依据基因序列, 分别设计10条和12条引物, 通过PCR重叠延伸法得到包含随机突变蛋白BBP的基因序列, 并重组到噬菌粒载体pCANTAB5E中构建Lipocalin突变文库, 库容达到 $4.0 \times 10^9$ . 以偶联分子BSA-克百威和OVA-克百威为靶分子, 采用柱式和平皿式交叉法对Lipocalin文库进行了筛选, 用竞争洗脱法洗脱特异结合活性的噬菌体. 经过3轮筛选, 从第3轮的洗脱液中随机挑选了10个重组克隆, 用Dot-blotting法检测出K7 anticalin分子能与克百威特异结合. 为研发克百威的Anticalin类检测试剂盒提供了候选分子, 也为拓宽Lipocalin文库在有害小分子检测方面的应用奠定了基础.

关键词: Lipocalin文库 克百威 Anticalin分子

Construction of Phage-displayed Library Based on the Lipocalin Scaffold and Screening Anticalins with Specificity for Carbofuran

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Abstract:

Carbofuran, which is used as toxic carbonate pesticide of crops, is highly toxic and small molecule. To detect the remainder of carbofuran efficiently is important to our healthy. The lipocalins, a family of diverse proteins that normally serve for the storage or transport of mostly hydrophobin and/or chemically sensitive organic compounds, can be reshaped by combinatorial protein design such that it recognizes small molecules. Sixteen residues at the center of binding site of the bilin-binding protein (BBP) from *Pieris brassicae* were subjected to random mutagenesis by overlapping PCR with 12 primers. Phagemid expression vector of pCANTAB5E was used to construct the lipocalin library. The library repotire is  $4.0 \times 10^9$ . BSA and OVA conjugated carbofuran respectively as the targeted protein, the library

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was screened with column and plate as supporter. After 3 rounds of panning, 10 phage clones were random selected with competitive elution. Dot-blotting showed that phage displayed anticalin, named as K7, bound carbofuran specifically among them. K7 can be used as potential anticalin to detect carbofuran of crops.

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Keywords: Lipocalin library Carbofuran Anticalin molecule

收稿日期 2007-06-18 修回日期 1900-01-01 网络版发布日期

DOI:

基金项目:

通讯作者: 孙远明

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#### 参考文献:

1. Bocskei Z., Groom C. R., Flower D. R., *et al.*. Nature[J], 1992, 360(6400): 186—188
2. Bianchet M. A., Bains G., Pelosi P., *et al.*. Nature Struct. Biol.[J], 1996, 3(11): 934—939
3. Newcomer M. E., Jones T. A., Aquist J., *et al.*. EMBO J.[J], 1984, 3(7): 1451—1454
4. Flower D. R., North A. C., Sansom C. E.. Biochim. Biophys. Acta[J], 2000, 1482(1/2): 9—24
5. Kramer K., Hock B.. Analy. Bioanal. Chem.[J], 2003, 377(3): 417—426
6. Kramer K.. J. Immunological Methods[J], 2002, 266(1/2): 209—220
7. Schlehuber S., Beste G., Skerra A.. J. Mol. Biol.[J], 2000, 297(5): 1105—1120
8. Beste G., Schmidt F. S., Stibora T., *et al.*. Proc. Natl. Acad. Sci. USA[J], 1999, 96(5): 1898—1903
9. Mercader J. V., Skerra A.. Analytical Biochemistry[J], 2002, 308(2): 269—277
10. Vogt M., Skerra A.. Chem. Bio. Chem.[J], 2004, 5(2): 191—199
11. Vopel S., Muhlbach H., Skerra A.. Biol. Chem.[J], 2005, 386(11): 1097—1104

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