

Selection and Production of Recombinant Binders for Use in Protein Microarrays



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(Quinton Balistreri)

SELECTION AND PRODUCTION OF RECOMBINANT BINDERS FOR USE IN PROTEIN MICROARRAYS



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GRIN Verlag Sep 2011, 2011. Taschenbuch. Book Condition: Neu. 211x146x10 mm. This item is printed on demand - Print on Demand Neuware - Diploma Thesis from the year 2009 in the subject Biology - Micro- and Molecular Biology, grade: 1,1, German Center of Cancer Research Heidelberg, language: English, abstract: Antibodies are essential everyday tools for molecular biologists and much depends on their performance and availability. Large numbers of antibodies are available already, however, these cover only a small part of the known proteome. The generation of antibodies for proteome-wide studies using conventional methods, which are based on animal immunisation, would be hardly feasible and not sustainable. Recombinant binders may be able to fill the gap of missing affinity reagents. The main advantages of recombinant binders are (1) the wide availability in form of fully synthetic DNA libraries (2) the independence on animal immunisation, which still is the main source of antibodies today and (3) the possibility to design completely new classes of synthetic binders with defined properties. The aim of this work was to establish the selection and purification of recombinant binders for use in protein microarrays. Binders were selected from a synthetic library of single chain fragment variable (scFv) antibodies using phage display as tool for selection. A number of binders against gamma globulins and BSA-FITC could be selected from the library and were subsequently used for establishment of downstream applications. Several methods for expression and purification of the binders were tested mostly in regard to scFv yield. Methods for detection were evaluated for their sensitivity and specificity. As the long term aim was the setup of a protein microarray based on recombinant binders, multiple immobilisation strategies were evaluated, including a one step on-chip purification and immobilisation of scFvs from crude expression samples. The on-chip purification was shown to...



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