The expansion of the hapten binding antibody library

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Introduction

Haptens are small molecules which cause an immune response only if they bind to a bigger conjugate. The produced antibodies then bind even to the unconjugated haptens. Antibody libraries are large collections of antibodies that can be searched to provide antibodies to various antigens speeding up the antibody discovery process. Antibodies can be obtained from the libraries directly to haptens without any conjugation to a carrier protein. At the University of Turku, the scFvM library based on human KV3-20 and HV3-23 in the framework has been made for hapten binding development (Huovinen et.al., 2012).

The goal

The goal of the project is to expand the existing hapten-binding antibody gene library by introducing a new light chain framework KV2-28, with cavity structure. The library utilizes the p3-phage display method **(fig.1)**.

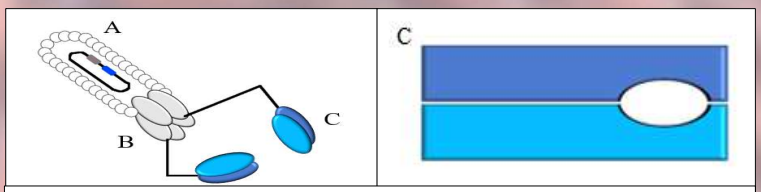

***Figure 1**: The structure of a p3-phagemid, used in the antibody library. The phagmid contains the p3 fusion protein gene (A). At the tip of the phagemids phage coat protein (B), there is a single chain fragment variable (scFv) with a cavity structure (C), which contains 6 different complementary determining regions (CDR) for specific hapten binding. This structure is the basis for phage display, used for scFv interactions examinations.*

Materials and methods

The hapten binding library was constructed using the KV2-28 gene with the human scFv backbone as a template. Sequence diversity was added to selected position at the CDR-L1, -L2 and -L3 loops **(fig.4)**. To eliminate out-of frame sequences the variable light (VL) library was purified with carbenicillin selection, after which it is then combined with the variable heavy (VH) amplified by PCR from the current M library for greater diversity. After the chains have been combined, the library is then characterized **(fig.2)**. The library is done using FASTR technique **(fig.3)**. The library consists of 10^9-10^{10} antibodies, with only one CDR-H3 loop size, unlike in the main library.

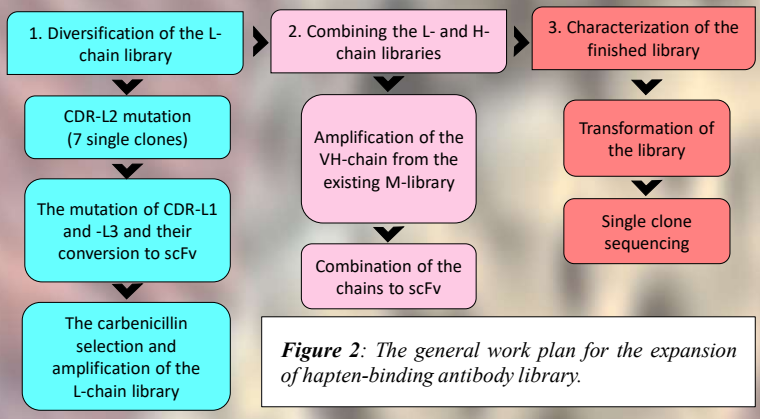

***Figure 2**: The general work plan for the expansion of hapten-binding antibody library.*

Results

The measured output of the VL-chain is 5.22 x 10^8 colony forming units (cfu). The diversity of the library is more than enough, with 77679-fold cell diversity compared to the theoretical diversity of 6720. Based on Sangers sequencing and after carbenicillin selection, 75% of the 47 VL clones are successful, with all of the intended mutations **(fig.4)**. The output of the combined KV2-28 and HV3-23 library is 1.33 x 10^{11} cfu, which indicates the library production being more efficient than usual. Of 18 clones which were successfully sequenced, 83% were correct annd in-frame.

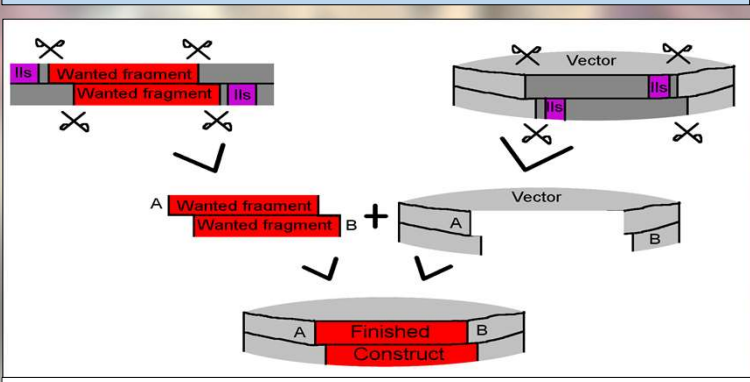

***Figure 3**: FASTR is a single-step procedure with simultaneous ligation and digestion. A specific recognition site of IIs restriction enzyme is added with primers in PCR. The IIs type enzyme cleaves outside the recognition sequence removing the site and leaves an intact gene for ligation. Since the ligation product does not have the IIs recognition site, this method drives the equilibrium of the reaction towards the product.*

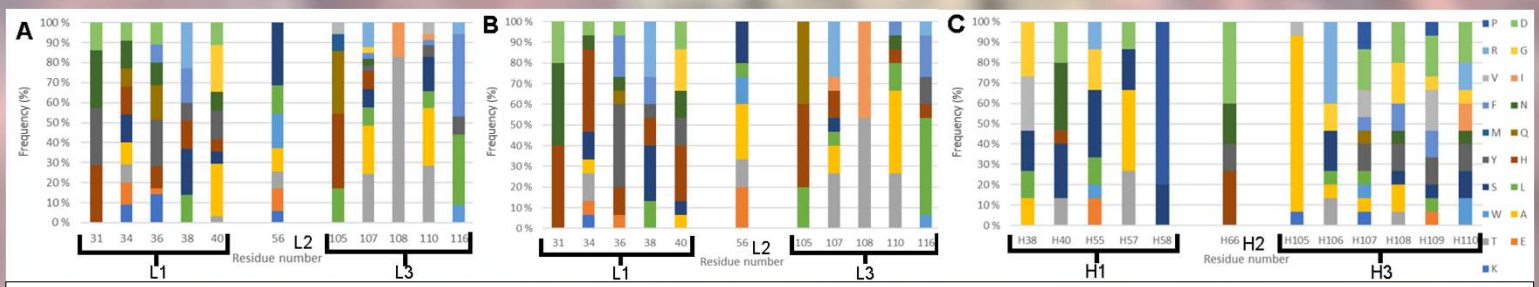

***Figure 4**: The Sangers sequencing result of antibody library clones. Colors correspond to specific amino acids, and the likelihood of the specific amino acid found at the planned part of the CDR-sequence is depicted. (A) The complete KV2-28 library. All the planned mutations in each CDR are present. (B) The light chain CDR-domains of the combined KV2-28 and HV2-23 library. The amino acid diversity is mostly unchanged. (C) The heavy chain CDR-domains of the combined KV2-28 and HV2-23 library. The diversity of the regions was not as diverse as planned.*

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Conclusions

The heavy and light chain libraries were successfully made into one insert with FASTR. The ligation and the transformation of the scFv-library were successful. All of the designed mutations were present at the planned residues, without any unexpected changes. Further testing is required for the phage display.