PureSelect2 - novel phage display platform for *in vitro* selection of therapeutic antibody fragments

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In vitro selection of therapeutic antibodies offers great alternative to classic *in vivo* approaches, especially in non-standard, more demanding cases. Here, we propose novel phage display-based platform for selection and engineering of antibody fragments, comprising of unique set of components indispensable for its enhanced functionality (i.e. vectors, *E.coli* strains, technology).

Improved display quality enabling efficient selection without nonspecific selective pressure

Historically, pVIII and pIII were the two main coat proteins of filamentous phage most frequently used for protein display purposes, resulting in development of multiple phage display libraries based on their use. However, both display formats have their own limitations. In case of pVIII, libraries tend to generate weaker binders, since apparent affinity to the molecular target is increased by "additive" binding strength of multiple recombinant pVIII proteins present on the phage surface (~ 2800 copies per each phage particle)[1,2].
On the other hand, since wild-type pIII is necessary for phage's efficient *E.coli* infection, helper phage-phagemid system was introduced, resulting in presentation of maximum one polypeptide copy per phage particle, which allows for selection of the strongest binders. However, during amplification of phage particles vast majority of phages do not present any polypeptide at all. Additionally, these particles are the most potent ones in infection, which leads to selection bias[1,2].
To circumvent those disadvantages, we propose library of antibody fragments presented on phage protein not directly involved in the infection process. Such strategy increases the long-term quality and effectivity of the library, preventing from nonspecific selection bias related to decreased infective potential of peptide-presenting phages.

Improved quality of the library obtained via elimination of "empty phages"

In order to obtain a fully functional library with each phage particle presenting an scFv, yet maintain control over phage proliferation, we constructed helper phage with deletion of chosen protein (**Fig.1**).
We also introduced genetically modified *E.coli* strain as part of our PureSelect2 platform. This strain with modified genome constitutively produces wild-type protein, complementing gene deletion in the M13KO7 genome and enabling its proliferation in the absence of recombinant fusion protein from the phagemid (**Fig.2**).

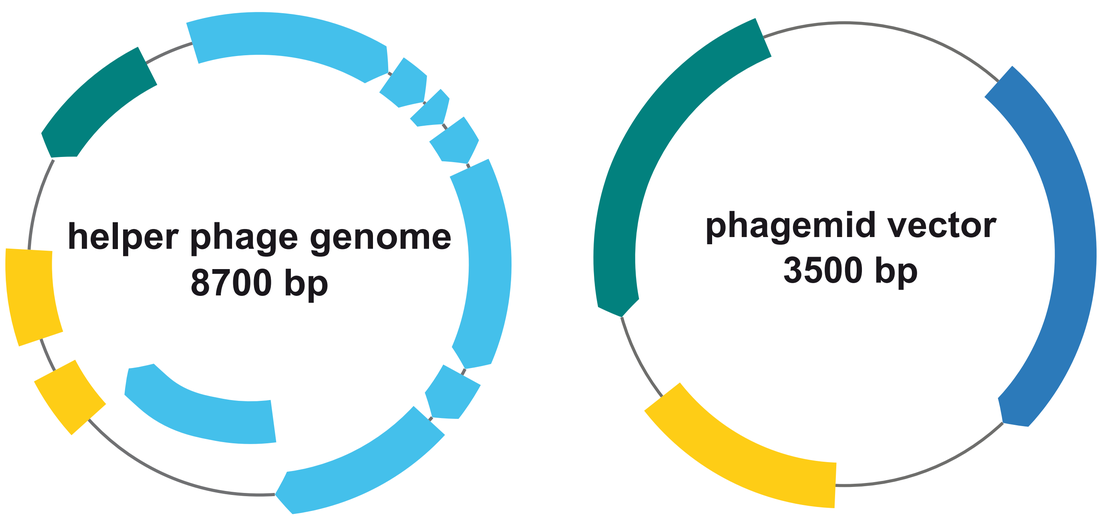

Fig.1 - The genomic map of designed vectors - helper phage (left), phagemid (right).

Functionality-based screening protocol to achieve promising candidates faster

We designed high-throughput cloning protocol to quickly clone the pool of variants after last round of selection into an optimized expression vector. After the cloning, only plasmids with the proper insert, selected due to the use of selective marker in the destination vector, will be successfully propagated in *E.coli* (**Fig.3**).
Finally, we also introduced changes facilitating screening and production of antibody fragments. The selected variants will be click-cloned into range of possible vectors and formats. This step enables us to overcome limitations related to phagemid vector, which is not designed to efficiently produce recombinant proteins. Moreover, library screening will not require screening in phage format followed by transfection of clones into non-amber suppressor strain, as well as will provide additional benefit of ready-to-express variants immediately after the first screening.

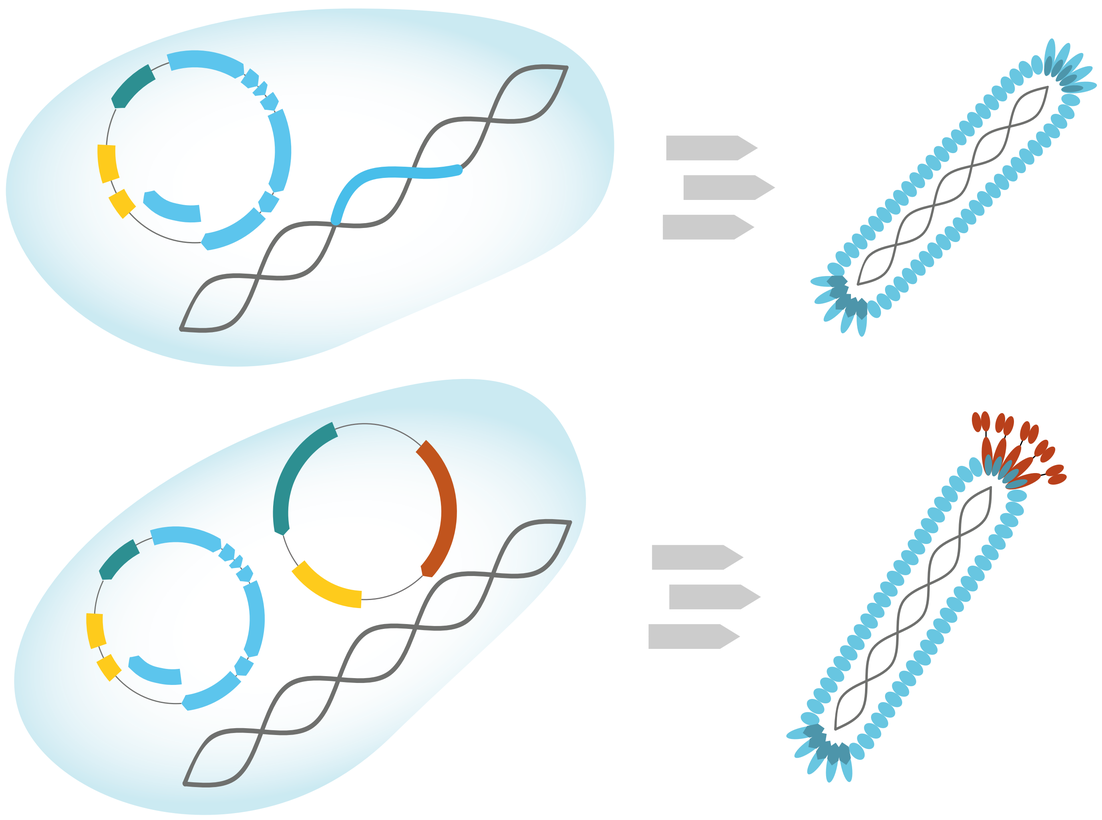

Fig.2 - The complementation of deleted protein gene either by recombinant E.coli or phagemid.

Internal positive control for selection on recombinant protein

For this purpose we designed dual vector system for expression of recombinant proteins. This system allows for efficient production of either tagged or untagged antigen of interest (AOI). Untagged version is available for phage display selection, while tagged one is used for screening procedure and enables us to include positive control in each experiment (even if there are no commercial antibodies available against the AOI). The positive control sample uses our proprietary antibody recognizing small peptide tag.

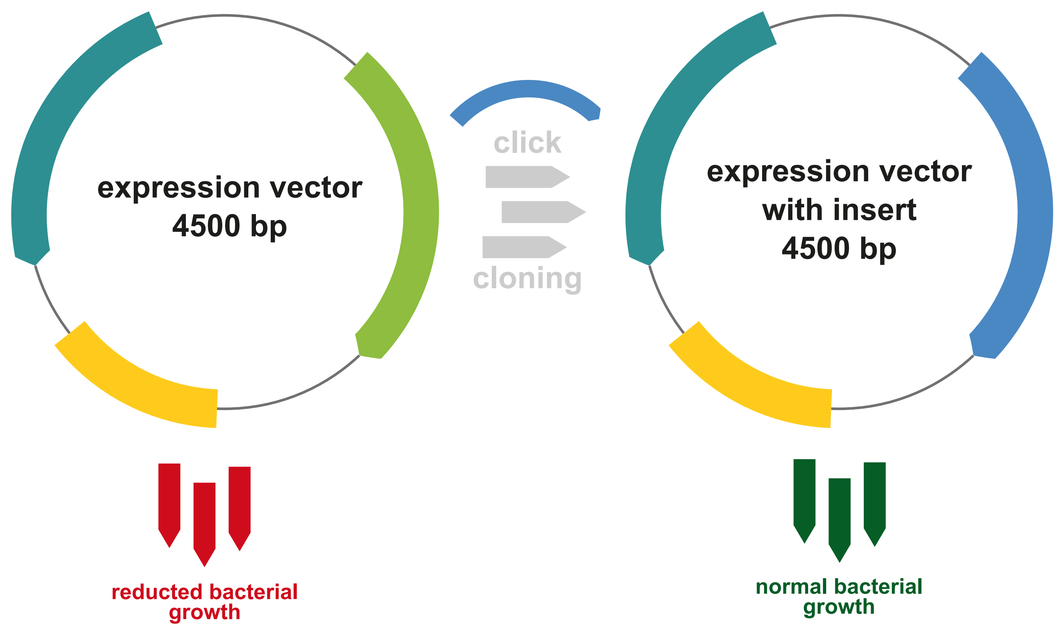

Fig.3 Selecion gene for improved efficiency of cloning scFv gene into an optimized expression vector.

One-step, faster phage purification during selection procedure

In order to quickly and gently purify phage particles, we introduced specific protein tag in the fusion protein. Obtained phage particles are purified in one-step affinity chromatography followed by phage titering in plate assay (to assess the retention of infectivity). Overall, procedure has been shortened to 1.5 hr, instead of 5 hr of phage precipitation with standard protocol. This simple step also allows to obtain "concentrated" pool of phage particles, with 100% of them presenting fusion protein on the surface.

Conclusions

We propose phage display platform of excellent quality ensured by modified display type, *E.coli* strain enabling highly efficient presentation of scFv fragments, as well as modified procedures of screening and phage purification allowing for faster procedure.

[1] Diane J. Rodi, Suneeta Mandava, and Lee Makowski. Filamentous Bacteriophage Structure and Biology. In: Sidhu Clarence Ronald S, editor. Phage Display in Biotechnology and Drug Discovery. 2005.
[2] Phage Display: Laboratory Manual. Carlos F. Barbas III , Dennis R. Burton , Jamie K. Scott , Gregg J. Silverman | The Quarterly Review of Biology: Vol 76, No 4. University of Chicago Press; 22 Oct 2015

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