**Production And Purification Of M13K07 Filamentous Phage In The Screening Of Single-domain Antibodies (Nanobody**®**) Against Multidrug-resistant Bacteria**

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**Background and aims.** Single-domain antibodies (nanobodies) are emerging as a potential alternative to traditional antibiotics in the fight against multidrug-resistant bacteria. Along with that, phage display technique has been widely applied to obtain synthetic nanobodies (sybodies) specific to various antigens of target bacteria from large gene libraries. This study focuses on optimizing the production and purification process of M13K07 filamentous phage (Helper phage) to harvest a high yield of phages carrying the sybody sequencing library, ready for biopanning.

**Methods.** The *E. coli* SS320 competent cells were electroporated with phagemid pDX\_init (Addgene #110101) carrying the gene encoding for the sybody against the maltose binding protein (MBP) found in the periplasmic space of cells, fused with phage’s protein pIII. The recombinant *E. coli* SS320 cells were infected with Helper Phage to package the vector. Phages were then purified applying the protein precipitation method using polyethylene glycol (PEG). The phage concentration was determined using the plaque assay method and real-time PCR. Successful expression on phage’s surface of the 6xHis-tag fusion sybody as well as the specific binding level between the sybody and MBP was determined using the ELISA method.

**Results.** The volume of 100 ml of culture delivered a phage concentration of 3 - 5 x 106 pfu/ml and 4 - 5 x 106 phagemid copies/ml. The ELISA test demonstrated successful expression of the 6xHis-tagged sybody and the specific binding level between the anti-MBP sybody-phage complex and MBP reached an IC50 value of 1.01 μg/ml, proving the potential use of phages in biopanning. SDS-PAGE also indicated a 60 kDa band of the anti-MBP sybody-phage complex.

**Conclusion.** The production and purification methods using PEG precipitation have provided a significant amount of phage with high concentration and purity, which is crucial for minimizing background signal during the screening of specific sybodies.

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