

ORT_32 - Construction of combinatorial libraries of human antibodies using Phage Display technology

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Introduction: The development of therapeutic antibodies is extremely important for oncological, infectious treatment and immunological check point. Antibodies can be developed predominantly through hybridoma technology followed by humanization or through phage display. Phage display technology, which is based on the use of filamentous bacteriophages that incorporate exogenous genes with diversity to compose a library and presents as a great alternative for the development of fully human antibodies. The main advantages of this platform are the generation of a repertoire of non-natural human antibodies, independent of the in vivo immune response, being applied to obtain mAbs against any type of antigen, such as self-antigens, toxic, unstable and non-immunogenic antigens.

Objectives: Synthesize libraries of human antibody fragments single-chain fragment (ScFv) and fragment antigen-binding (Fab).

Methodology: Scfv and Fab libraries were constructed from the isolation of cDNA from RNA (peripheral blood) from blood bank donors (100 individuals) and individuals recovered and tested positive for COVID-19 (22 individuals). Degenerate primers established by Barbas et al 2001 were used on PCRs. To construct the scFv, we performed six amplifications using sense primers for variable chains (heavy and light) combined with antisense for the kappa region, finishing with a set of primers for PCR overlap scFv. For the Fab fragment, amplification of the constant chain regions was carried out using the pComb3XTT vector, in addition to PCR overlap steps converging the heavy chain and light chain followed by the last step of PCR overlap, converging the entire Fab fragment. Next, the fragments and pComb 3XTT vector were digested, ligated and transformed into the library.

Results: Two ScFv libraries (Covid-19 and Naïve) and one Fab library (Covid-19) were constructed using transcripts from blood bank donors and patients recovered from Covid-19.

Conclusion: The technology for constructing libraries of antibody fragments by Phage Display is the starting point for generating antibodies against specific targets to assist in the diagnosis and treatment of various diseases and represent an immense possibility of obtaining therapeutic antibodies in a short period of time, helping to combat possible epidemics and/or pandemics.

Keywords: Phage display; Antibodies