

P015 Affinity maturation of phage display antibody populations using ribosome display

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Selections were performed using ribosome display with reducing concentrations of the antigen, insulin, to 1nM, on antibody populations derived from three rounds of phage display selections. These ribosome display stringency selections were performed with or without error prone mutagenesis between rounds of selection and compared with the output of phage display selections at the same concentration. Whereas phage display generated an antibody, D3, with K_d of 5.8nM as a scFv fragment, ribosome display generated higher affinity variants of this antibody with K_d values of 189pM and 152pM, without or with the use of error prone mutagenesis respectively. The affinities were increased for each antibody on conversion of the scFv fragments to whole IgG format, but the relative affinities were retained as illustrated by increased sensitivity in an ELISA using detection with the ribosome display derived variant PSEP010(1)H7IgG4. Mutation of D101 of D3 to glycine or valine, removing the salt bridge between K94 and D101 at the base of VHCDR3, was responsible for the enhanced sensitivity observed. In addition to the variants of D3, other unrelated antibodies of comparable or higher affinity for insulin, were isolated by ribosome display, but not phage display. Affinity maturation of phage antibody populations using ribosome display is a valuable method of rapidly generating diverse, higher affinity antibodies to antigen.