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# Construction of a semisynthetic antibody library using trinucleotide oligos

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## ABSTRACT

**A semisynthetic antibody library composed of single chain Fv fragments (scFv) was constructed by replacing the heavy chain CDR3 region of a human scFv by a random sequence of eight amino acids using trinucleotide codons. After cloning into a phage display vector, an antibody library was generated with a complexity of $8 \times 10^8$ independent clones. The library was screened for binders to dinitrophenol, fluorescein isothiocyanate and 3-nitro-4-hydroxy-5-iodophenyl-acetic acid. scFv antibodies that specifically bound the antigen were obtained in each case.**

Recombinant antibody technology has proven to be a valuable tool for generating antibodies to a wide variety of antigens (1–6). In most of the published systems, an antibody library is presented on the surface of a filamentous phage and clones with a desired specificity are enriched by a panning procedure. The antibody repertoires of these libraries are usually derived from the IgG or IgM repertoires of human or murine sources. In a more recent approach, the repertoire has been generated by introducing random sequences mainly into the CDR3 region of the heavy chain (7–9). This method can generate highly complex libraries and faciliate the selection of antibodies against self-antigens, which are normally removed by the negative selection of the immune system.

One limitation of semisynthetic libraries is the composition of random sequences introduced by conventional techniques. The widely used codon NNK for random amino acids gives an unequal distribution of the 20 amino acids and also results in 3% stop codons. To circumvent both problems, we constructed a library in which the CDR3 region of the heavy chain was encoded by a random 24 bp oligonucleotide sequence synthesized from a mixture of presynthesized codons (10). The codon mixture was chosen such that there was an equal opportunity that each position could be occupied by any amino acid except cysteine and stop codons. For cloning purposes, the oligo was synthesized with suitable flanking sequences on both ends.

The library was constructed as outlined in Figure 1. In a first step the 'random oligo' 5′-GACACGGCCGTGTATTACTGTGTGA-GA(Tri)$_8$ *TGGGGCAAA*GGGACCACGGTC-3′ was elongated with primer 'rnd back': 5′-TTCTTCAAGCTTTGGGGCGGAT-GCACTCCCTGAGGAGACGGT*GACCGTGGTCCCTTTGCCC-*

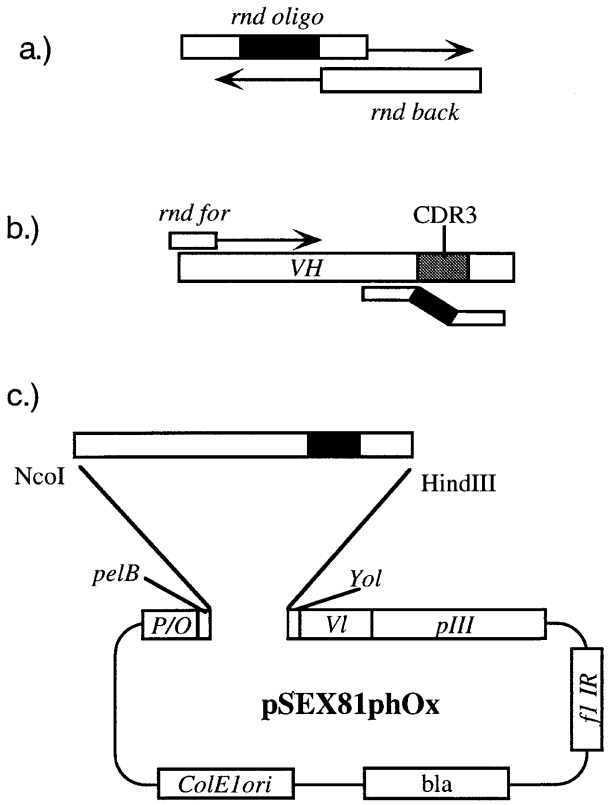


**Figure 1.** Cloning of a semisynthetic library. (**a**) Elongation reaction; (**b**) PCR for generation of the library using a model antibody as template; (**c**) cloning of the library in pSEX 81-phOx. *Rnd oligo, rnd back, rnd for*, oligos described in the text; *VH*, variable domain of the heavy chain of a human antibody against phOx; *VL*, variable domain of the light chain of a human antibody against phOx; *Yol*, Peptide linker of 18 amino acids between VH and VL, including an epitope recognized by the monoclonal antibody YOL 1/34; *pIII*, sequence encoding the gene III product of bacteriophage M13. *f1 IR*, intergenic region of phage f1; *bla*, β-lactamase gene; *ColE1ori*, origin of replication of ColE1; *P/O*, lac operon wt promoter/operator; *pelB*, signal peptide of pectate lyase of *Erwinia carotovora*.

*CA*-3′ (overlapping regions in italics) in a PCR-like reaction [400 pmol of each primer, 250 µM of each nucleotide, 10 U PCR-Expand (Boehringer Mannheim) in a total volume of 200 µl,

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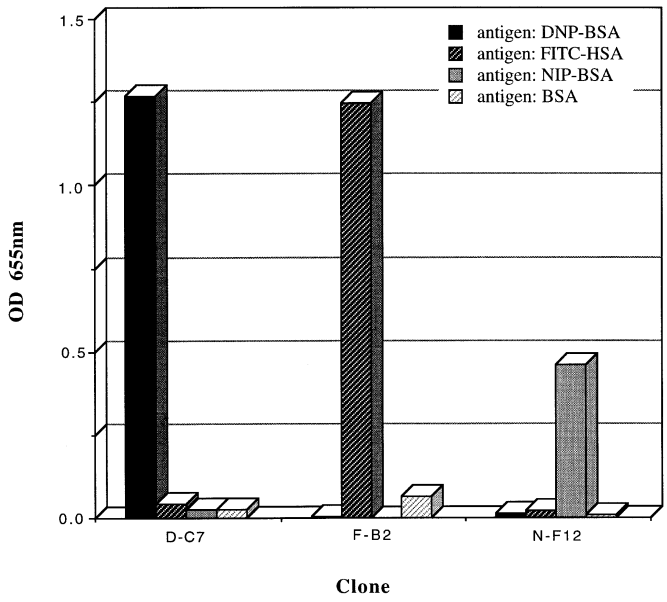


**Figure 2.** Binding specificity of selected clones obtained after screening the semisynthetic library as determined by phage-ELISA. Selected clones obtained after the third round of panning are shown (fourth round of screening for FITC-HSA). Clone D-C7 was obtained after screening on DNP-BSA, clone F-B2 after screening on FITC-HSA and clone N-F12 after screening on NIP-BSA.

five cycles: 94°C, 15 s; 55°C, 30 s; 72°C, 60 s]. The product was gel-purified and used together with primer 'rnd for': CAGCCG-GCCATGGCCCAGGTG in a PCR reaction [40 pmol of each primer, 250 µM of each nucleotide, 10 U PCR-Expand (Boehringer Mannheim) in a total volume of 200 µl, 20 cycles: 94°C, 15 s; 55°C, 30 s; 72°C, 60 s]. The template for this reaction was the heavy chain variable domain of a human antibody against phOx (3). The PCR product was gel-purified, digested with *Nco*I–*Hin*dIII (Boehringer Mannheim) and substituted for the heavy chain variable region of the phage display vector pSEX 81-phOx (5). After transforming *Escherichia coli* XL1-blue (Stratagene, LaJolla), an antibody phagemid library was obtained with a complexity of $8 \times 10^8$ independent clones. The library was screened with the three haptens dinitrophenol (DNP), fluorescein isothiocyanate (FITC) and 3-nitro-4-hydroxy-5-iodophenylacetic acid (NIP) conjugated to bovine serum albumin (DNP, NIP) or human serum albumin (FITC), respectively. Specific clones were selected following a procedure described elsewhere (5); 25 µg antigen was used in each panning round. After three rounds of panning (four rounds for FITC-HSA) 48 single clones from each panning experiment were picked and analysed. The specificity of binding was checked by phage-ELISA as described elsewhere (5,6). Thirty eight clones bound specifically to NIP-BSA, 10 to FITC-HSA and 21 to DNP-BSA. Examples are shown in Figure 2.

The presynthesized codons facilitate the generation of a new class of random sequences. In contrast to NNK randomized sequences, a precise ratio of amino acids can be readily achieved. A codon usage can be also chosen that is best suited for the host organism. By varying the mixture of the 20 trinucleotides, it is possible to design a well defined mixture of codons for each position. A controlled bias can be achieved towards or against specific amino acids or classes of amino acids; some can be completely excluded and stop codons avoided. This could significantly increase the chances of selecting a functional antibody. The same advantages apply to peptide libraries. The trinucleotide approach is not limited to antibody or peptide libraries and can be employed in any area of research where protein mutagenesis is required. For example, computer models of protein–ligand binding can be used as a basis for the limited randomization of key amino acids with mixtures of amino acids that have a particular range of desired characteristics.

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