



(11) **EP 1 300 419 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:  
**13.06.2007 Bulletin 2007/24**

(51) Int Cl.:  
**C07K 16/28** (2006.01) **A61K 39/395** (2006.01)  
**G01N 33/53** (2006.01) **C12N 15/13** (2006.01)  
**C12N 5/10** (2006.01) **C12N 15/63** (2006.01)

(21) Application number: **01123851.6**

(22) Date of filing: **05.10.2001**

(54) **Antibody of human origin for inhibiting thrombocyte aggregation**

Antikörper menschlichen Ursprungs zur Hemmung der Thrombozytenaggregation

Anticorps d'origine humaine pour l'inhibition de l'agrégation des thrombocytes

(84) Designated Contracting States:  
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR**

(43) Date of publication of application:  
**09.04.2003 Bulletin 2003/15**

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(56) References cited:  
• **NGUYEN-HO P ET AL: "Platelet glycoprotein IIb / IIIa receptor antagonists and coronary artery disease." CURR ATHEROSCLER REP, (2001 MAR) 3 (2) 139-48. REF: 56 , XP001057096**

- **BERKOWITZ S D: "Current knowledge of the platelet glycoprotein IIb / IIIa receptor antagonists for the treatment of coronary artery disease." HAEMOSTASIS, (2000) 30 SUPPL 3 27-43. REF: 63 , XP001057638**
- **DOBESH P P ET AL: "Advancing the battle against acute ischemic syndromes: a focus on the GP IIb-IIIa inhibitors." PHARMACOTHERAPY, (1998 JUL-AUG) 18 (4) 663-85. REF: 91 , XP001057099**
- **JUBELIRER S J ET AL: "Acute profound thrombocytopenia following C7E3 Fab (Abciximab) therapy: case reports, review of the literature and implications for therapy." AMERICAN JOURNAL OF HEMATOLOGY, (1999 JUL) 61 (3) 205-8. REF: 11 , XP001057619**
- **SUZUKI K ET AL: "Comparison of the antiplatelet effect of YM337 and abciximab in rhesus monkeys." EUROPEAN JOURNAL OF PHARMACOLOGY, (1997 OCT 8) 336 (2-3) 169-76. , XP001057621**
- **SUZUKI ET AL: "Comparative studies of a Humanized Anti-glycoprotein IIb/IIIa monoclonal antibody, YM337, and abciximab on in vitro antiplatelet effect and binding properties" BIOL. PHARM. BULL., vol. 25, no. 8, 2002, pages 1006-1012,**

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

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**Description**

**[0001]** The present invention is directed to an antibody for inhibiting platelet aggregation, and a method for identifying and/or isolating such an antibody. Furthermore, the present invention concerns the DNA coding for this antibody and a pharmaceutical or diagnostic preparation containing the antibody or its coding DNA.

**[0002]** Platelets or thrombocytes play a crucial role in the field of thrombosis, myocardial infarction and unstable angina: The platelet integrin receptor GPIIb/IIIa is of particular importance since it mediates platelet aggregation by binding of the bivalent plasma molecule fibrinogen. This receptor has at least two conformational states: 1) A non-activated state, which is the default state on unstimulated platelets. In this non-activated state the receptor demonstrates a very low affinity for its ligands and is not capable of inducing platelet aggregation. 2) An activated state which is present after platelet activation, e.g. by thrombin. In this activated state GPIIb/IIIa has undergone a conformational change, which leads to high affinity binding of fibrinogen (Shatill et al., J. Biol. Chem. 1985: 260(20): 11107-11114).

**[0003]** Consequently the therapeutic blockade of GPIIb/IIIa is a very effective anti-platelet strategy, since it affects the final common endpoint of the platelet activation cascade. During the last years a great variety of GPIIb/IIIa-blockers have been developed. These are either chimeric mouse/human Fab-fragments of a GPIIb/IIIa-blocking monoclonal antibody (Abciximab) (Coller B., et al., J. Clin. Invest. 1983, 72: 325-338), cyclic peptides (Eptifibatide) or polycyclic synthetic peptidomimetics (e.g. Tirofiban) (Bhatt DL and Topol EJ. JAMA. 2000; 284(12):1549-58; Topol EJ, et al., Lancet. 1999; 353(9148):227-31). This therapy has been proved to be effective but there still retain some problems in this context:

- especially under the therapy with Abciximab, an increased prevalence of severe thrombocytopenia is present (~1%) (Dasgupta H., et al., Am Heart J. 2000;140(2):206-11).
- due to the expensive production the costs of the therapy are considerably high, especially for Abciximab. (Hillegass WB, et al., Pharmacoeconomics. 2001; 19(1):41-55).
- there is an increase in bleeding complications which are especially important when GPIIb/IIIa-blockers are combined with thrombolysis.
- synthetic GPIIb/IIIa-blockers which are administered orally brought disappointing results, due to their pharmacokinetic properties, particularly a rather low affinity for the receptor. (Chew DP. et al., Circulation. 2001,103(2):201-206).
- there is evidence that GPIIb/IIIa-blocker, especially the low molecular agents, interact with the receptor after binding. This might result in a paradoxical intrinsic activating effect (Peter K., et al., Blood. 1998; 92 (9) : 3290-)
- reversibility of the effect of Abciximab is very slow (>12 hours)
- approx. 6% of the patients treated with Abciximab develop anti-human-chimeric antibodies (AHAC); 11% in case of patients treated repeatedly (Gawaz M., Therapie bei koronarer Herzerkrankung. Stuttgart, New York: Thieme, 1999).

**[0004]** All GPIIb/IIIa-blockers, currently used, are binding to the activated and non-activated receptor with similar affinity. An activation specific inhibitor might offer several advantages. For example platelet adhesion would still be intact which should result in a reduction of bleeding events. Moreover interactions with the non-activated receptor would be prevented. It would be desirable to develop a smaller GPIIb/IIIa-blocking agent with an affinity similar to an antibody, which should demonstrate better pharmacokinetic properties.

**[0005]** Another application for an activation specific antibody would be the detection of activated platelets, which is very useful in a variety of research and diagnostic-settings.

**[0006]** It is therefore the object of the present invention to find an antibody with such improved properties, as well as to provide methods for identifying such an antibody.

**[0007]** This object is solved by providing the antibody according to independent claim 1. Further advantageous features, embodiments and aspects of the present invention will become more readily understandable when looking at the further independent and dependent claims, the description and the drawings.

**[0008]** Accordingly, the invention is directed to an antibody of human origin for inhibiting platelet aggregation, characterised in that it is effective by substantially exclusive binding to the activated state of platelet integrin receptor GPIIb/IIIa.

**[0009]** The terms thrombocyte and platelet are used synonymously in this specification. The general term "platelet integrin receptor" means "platelet integrin receptor GPIIb/IIIa".

**[0010]** According to the present invention the antibody binds to the platelet integrin receptor GPIIb/IIIa (alpha IIb/beta 3) and inhibits the binding of the natural ligand fibrogen. As detailed above, this receptor is characterised by inducing the aggregation process when fibrinogen binds to it. Through blocking this receptor, crosslinking is impossible.

**[0011]** Due to the more selective effects obtainable, the antibody does "substantially exclusively bind" to the activated conformation of the platelet integrin receptor. This means that its binding affinity to the activated conformation of the platelet integrin receptor is much greater than its respective affinity for binding to the inactive conformation of the platelet integrin receptor. At best, the agent is substantially unable to bind to the nonactivated conformation of the integrin receptor.

**[0012]** In the present specification the term "antibody" means immunoglobulins of human origin. The immunoglobulin may be also a fragment of human immunoglobulins comprising the variable domains of the heavy and light chain. The fragment may be a single chain antibody fragment (scFv), Fab or recombinant constructs and derivatives thereof. It may be monovalent, bivalent or multivalent.

5 **[0013]** It can contain modifications to its amino acid sequence when compared to genuine antibodies and exhibit a modified domain structure. It must however, still be able to adopt the typical domain configuration found in native antibodies, as well as an amino acid sequence, which is able to bind to targets (antigens) with high specificity. Typical examples of antibodies derivatives are antibodies coupled to other polypeptides, rearranged antibody domains or fragments of antibodies. The antibody may also comprise at least one further compound, e.g. a protein domain, said protein domain being linked by covalent or non-covalent bonds. The linkage can be based on genetic fusion according to the methods known in the art. The additional domain present in the fusion protein comprising the antibody employed in accordance with the invention may preferably be linked by a flexible linker, advantageously a peptide linker, wherein said peptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of the further protein domain and the N-terminal end of the antibody or vice versa. The above mentioned fusion protein may further comprise a cleavable linker or cleavage site for proteinases. Thus, e.g., the antibody might be linked to an effector molecule having a conformation suitable for biological activity or selective binding to a solid support, a biologically active substance (e.g. a cytokine or growth hormone), a chemical agent, a peptide, a protein or a drug.

10 **[0014]** The antibody of the present invention is of human origin. This is a particularly important feature of the invention, since it opens the use of such antibodies to a therapy in human patients without the risk of adverse immune reactions against other "foreign" antibody types. In particular, the overall structure/sequence and the constant regions of the used antibody are of human origin. The source of the human antibody may be a phage display library of natural or modified human antibody fragments, screened for antibodies with affinity for thrombocytes.

15 **[0015]** Preferably, the antibody is a single chain antibody where a VH domain is linked to a VL domain. The term "linked" means preferably a peptide bond. Such a single chain antibody is preferably a recombinant scFv antibody. Methods for producing such a single chain antibody with the above mentioned properties or of DNA sequences coding for such an antibody, its expression in suitable hosts and its recovery and purification are described for examples in WO-A-89/09622, WO-A-89/01783, EP-A-0 239 400, WO90/07861 and Colcher et al., Cancer Research 49 (1989), p. 1732-1745. The scFv employed may be a recombinant construct of single chain antibody fragment(s), if such rearrangements or changes to the sequence are necessary in order to obtain the desired product. The person skilled in the art knows methods how to modify the respective immunoglobulin domains, e.g. via amino acid deletion, insertions, substitutions and/or recombinations. Methods for introducing such modifications in the coding sequence for the immunoglobulin chain are known to person skilled in the art (e.g. Sambrook et al., Molecular Cloning - A Laboratory Manual, Cold Spring Harbor (1989), N.Y.) On the other hand, the single chain antibody fragment may for example be derived from a human IgM or IgG antibody. Alternatively, recombinant BsAb or diabodies (containing two scFv fragments preferably linked via a peptide linker) can be formed. It will be also advantageously to construct tandem diabodies by homodimerisation of single chain fragments comprising four antibody variable domains (VH and VL) of two different specificities.

20 **[0016]** Due to the huge variability of the antibody generation process in the course of an immune response, in general a large number of different sequences suitable for attacking a foreign antigen can be produced. It is clear to the skilled person that therefore, several embodiments of antibody sequences could be found for meeting the requirements of the present invention. As an example, which is tested and worked well, the antibody according to the invention may be characterised in that the fragment comprises an amino acid region which comprises the translation product of the nucleic acid sequence of Fig. 2 (SEQ. No. 1). In a further preferred embodiment it comprises the amino acid sequence as shown in Fig. 2 or it consists of the amino acid sequence of Fig. 2. In a further embodiment, the present invention provides nucleic acid molecules encoding a fragment, derivative or allelic variation of the above polypeptide which have substantially the same properties as that of Fig. 2. The term "derivative" in this context means that the sequence of these molecules differ from the sequences of the nucleic acid molecules and/or of the amino acid sequence of Fig. 2 at one or several positions but have a high level of homology to these sequences. Homology hereby means a sequence identity of at least 60%, in particular an identity of at least 70 or 80%, preferably of more than 90% and particularly preferred of more than 95%. The deviations of the above-mentioned nucleic acid molecules or peptide molecules may have been produced by deletion, substitutions, insertions or recombination.

25 **[0017]** Another suitable example is a synthetic library of antibody sequences. The identified fragment comprises a heavy chain CDR3 domain which contains the sequence ELEAYCRGDCYPPYYG or a derivative thereof with comparable structure and properties. This sequence is found to be able of binding to the integrin receptor, maybe because it can mimic the fibrinogen structure.

30 **[0018]** A further preferred embodiment concerns the DNA sequence coding for the single chain antibody. These DNA sequences can be inserted into a vector or expression vector. Thus, the present invention also relates to vectors and expression vectors containing these DNA sequences. The term "vector" means a plasmid (pUC18, pBR322, pBlueScript,

etc.), a virus or any other suitable vehicle. In a preferred embodiment the DNA sequences are functionally linked to regulatory elements which allow their expression in procaryotic or eucaryotic host cells. Such vectors contain besides the regulatory elements (e.g. promoter) a replication origin and specific genes which allow the phenotypic selection of a transformed host cell. The regulatory elements for the expression in procaryotes (e.g. E. coli) are lac-, trp-promoter or T7 promoter, and for the expression in eucaryotes AOX1- or Gal promoter (for expression in yeasts) and CMV-, SV40-, RVS-40 promoter, CMV- or SV40 enhancer (for expression in animal cells). Further examples for promoters are metallothionein I and polyhderin promoter. Suitable expression vectors for E. coli are pGEMEX, pUC derivatives, pEXHAM and pGEX-2T. Suitable promoters for the expression in yeast are pY100 and Ycpad1 and for the expression in mammal cells pMSXND, pKCR, pEFBOS, cDM8 and pCEV4.

**[0019]** General methods known in the art can be used for the construction of the expression vectors which contain the DNA sequences of the present invention and suitable regulatory elements. Examples of these techniques are in-vitro recombination techniques, synthetic methods and in-vivo recombination techniques (c.f. Sambrook et al., Molecular Cloning - A Laboratory Manual, Cold Spring Harbor (1989), N.Y.). The DNA sequences according to the present invention may be also inserted into a vector in combination with DNA sequences coding for other proteins or peptides to be expressed as fusion proteins.

**[0020]** The present invention further concerns host cells containing these vectors. These host cells are e.g. bacteria (e.g. E. coli strains XL1blue, HB101, DH1, x1776, JM101, JM109, BL21 and SG13009), Yeasts (preferably S. cerevisiae), insect cells (preferably sf9 cells) and animal cells (preferably mammal cells). Preferred mammal cells are myeloma cells, preferably mouse myeloma cells). Methods for transforming these host cells, methods for the phenotypic selection of transformants and for the expression of the DNA sequences according to the present invention by using the aforementioned vectors are known in the present technical field.

**[0021]** The present invention further relates to a methods for the recombinant production of the (single chain) antibody by using the aforementioned expression vectors. This method comprises the cultivation of the aforementioned host cells under conditions which allow the expression (preferably stable expression) of the protein (or fusion protein) and the recovery of the protein from the culture or from the host cells. The person skilled in the art knows conditions how to cultivate transformed or transfected host cells. Suitable methods for the recombinant production of proteins are known (e.g. Holmgren, Annual Rev. Biochem. 54 (1985), 237; La Valle et al., Bio/Technology 11 (1993), 187, Wong, Curr. Opin. Biotech. 6 (1995), 517; Davies, Curr. Opin. Biotech 6 (1995), 543). Furthermore, suitable purification methods are known (e.g. preparative chromatographie, affinity chromatographie, HPLC etc.).

**[0022]** The invention is further directed to a process for identifying and/or isolating antibodies for inhibiting platelet aggregation by binding to the activated form of integrin receptor GPIIb/IIIa of blood thrombocytes.

**[0023]** Such process according to the invention comprises the following steps:

- providing a library of nucleic acids encoding for sequences of candidates;
- producing a phage library from said nucleic acids library;
- successively reacting said phage library with nonactive thrombocytes, active thrombocytes, other cells expressing nonactive integrin receptor molecules, and other cells expressing active integrin receptor molecules; and
- eluting phages bond to said thrombocytes or other cells expressing active integrin receptor molecules.

**[0024]** An important step of the inventive process is that the phage library is depleted of less suitable polypeptides, which either bind to nonactivated platelets, or to other components on the surface of activated platelets. Following each of the binding steps, a recovery of the selected phages should be performed, which can be done with known methods. Finally, those phages carrying polypeptides which specifically bind to the integrin receptor, are tested for their blocking activity.

**[0025]** The steps of selecting with other cells can be also omitted. By this modification, phages inhibiting platelet aggregation by other mechanisms may be detected.

**[0026]** By this, a "natural library", based on the antibody population of the donors, can be obtained

**[0027]** Alternatively, a synthetic library may be used, wherein the step of providing a library comprises the following steps:

- providing a nucleic acid containing a sequence for a single chain antibody fragment containing a heavy and a light variable domain; and
- introducing at least one randomised nucleotide sequence in a region of said single chain antibody fragment.

**[0028]** The region into which the at least one randomised nucleotide sequence is introduced, preferably is the CDR3 region of vH or vL such a scFv.

**[0029]** Said other cells may preferably be CHO cells, which are well known and may express the integrin receptor on their surface after having been transformed.

5 **[0030]** The invention is further directed to the use of a pharmaceutical composition containing the antibody, DNA or expression vectors according to the present invention for blocking the platelet integrin receptor on thrombocytes.

**[0031]** The invention is still further directed to the use of the antibody, DNA or expression vector according to the invention for manufacturing a pharmaceutical composition.

10 **[0032]** The subject matter of the present invention is also of diagnostic interest. It may be used for determining the number of activated thrombocytes in relation to non-activated thrombocytes in a patient. It is particular useful for monitoring the (de)activation status if the patient is treated with thrombocyte aggregation inhibitors.

**[0033]** The pharmaceutical or diagnostic composition may contain additionally a pharmaceutically acceptable carrier. Suitable carriers are phosphate buffered saline solutions, Water, emulsions (e.g. water-in-oil emulsions), surfactants, sterile solutions etc. The administration of the pharmaceutical composition may be orally or parenterally (e.g. topically, 15 intra-arterially, intramuscularly, subcutaneously, intramedullarly, intrathecally, intraventricularly, intravenously, intra-peritoneally or intranasally). The suitable dosage will be determined by the medical doctor and is dependent on various conditions (e.g. age, sex, weight of the patient, kind of illness and kind of administration, etc.).

**[0034]** The DNA sequences of the present invention may be also inserted into a vector suitable for gene therapy, e.g. under the control of a tissue-specific promoter. In a preferred embodiment the vector containing the DNA sequences is a virus (e.g. an adenovirus, vaccinia virus or adeno-associated virus). Preferred are retroviruses. Examples of suitable 20 retroviruses are MoMuLV, HaMuSV, MuMTV, RSV or GaLV. For gene therapy purposes the DNA sequences according to the present invention may be also transported in form of colloidal dispersions to the target cells. In this connection also liposomes and lipoplexes are mentioned (Mannino et al., Biotechniques 6 (1988), 682).

**[0035]** Finally, the invention is directed to a method a treating a patient, comprising the following step:

25 administering a pharmaceutical composition according to the invention in a pharmaceutically effective dose to the patient.

**[0036]** In the following, the invention shall be further detailed by exemplifying, non limiting embodiments, in which 30 reference will be made to the accompanying drawings:

Fig. 1 shows a FACS analysis of a clone expressing an antibody fragment according to a first embodiment of the invention;

35 Fig. 2a shows the nucleic acid sequence of clone MB9 coding for a scFV antibody according to the present invention; Fig. 2b shows the amino acid sequence of MB9.

Fig. 3 shows the sequence of C9 scFv and E4 scFv

40 Fig. 4 shows oligonucleotides used for the construction of the human scFv based synthetic library. BbsI restriction enzyme recognition sites are indicated in bold style, cut sites are underlined

Fig. 5 shows a schematic representation of annealing positions of oligonucleotides used for the construction of pEXHAM4/C9 and pEXHAM4/E4. Genes of the scFv's C9 and E4 cloned in pEXHAM1 are shown as boxes. Black 45 painted areas represent CDR regions; Oligonucleotides are represented by arrows and identified by numbers (c.f. Fig. 4). Bpil restriction endonuclease recognition sites are indicated.

Fig. 6 shows vector maps of pEXHAM4/C9 and pEXHAM4/E4

50 Fig. 7 shows vector maps of pEXHAM7/C9 and pEXHAM7/E4

Fig. 8 lists oligonucleotides used as primers in 1. PCR for amplification of human heavy and light chain variable regions

55 Fig. 9 lists oligonucleotides used as primers in 2 PCR for introduction of restriction endonuclease recognition sequences (Marked in bold style)

Fig. 10 shows the FACS analysis of clones SA8, SA10 and SA11. Binding of indicated scFv's to activated (black curve) and non-activated (grey curve) thrombocytes.

Fig. 11 shows the entire nucleotide sequence concerning the vector map pEXHAM4/E4.

Fig. 12 shows the entire nucleotide sequence concerning the vector map pEXHAM4/C9.

5 Fig. 13 shows the entire nucleotide sequence concerning the vector map pEXHAM7/E4.

Fig. 14 shows the entire nucleotide sequence concerning the vector map pEXHAM7/C9

10 **[0037]** In the following, examples for the production of human scFv antibodies specific for activated platelet integrin receptor GPIIb/IIIa will be given.

#### General strategy

15 **[0038]** Phage libraries for the display of single chain antibody fragments (scFv) are generated from human IgM antibody genes. Alternatively, a synthetic library is generated by randomisation of the CDR3 region of the heavy chain in two scFv master frameworks of human origin. Both libraries are substracted for not activation specific binders by incubation on resting thrombocytes prior to using them for selection on activated platelets. To focus the selection onto the GPIIbIIIa receptor additional rounds of selection are done on *in vitro* cultivated cells expressing recombinant GPIIbIIIa receptor. Following the selection scFv clones are analysed for binding to activated thrombocytes and competition of fibronogen binding by FACS analysis.

#### Example 1: Production of the human scFv antibody fragment MB9

##### RNA and cDNA preparation

25 **[0039]** Total RNA is isolated from spleen samples of six human donors and peripheral blood lymphocytes (PBL) of five healthy human donors (app.  $1\text{-}5 \times 10^8$  PBLs each, RNeasy (TM) Midiprep.Kit, Qiagen). From total RNA poly A<sup>+</sup>-RNA is prepared (Oligotex mRNA Kit, Qiagen) and used for cDNA synthesis (SuperScript™ Preamplifications System, Gibco BRL/LIFE Technologies).

##### Amplification of human Ig variable regions

35 **[0040]** Oligonucleotides used in PCR for amplification of variable regions of human immunoglobulin heavy and light chains those of Fig. 8. Heavy chains are amplified using a single IgM specific constant primer and one of a number of different primers (VH-1 to VH-7) specific for the variable region in separate PCR reactions. Accordingly lambda and kappa light chains are amplified using a single lambda or kappa specific constant primer and one out of a number of different variable primers (Vλ-1 to Vλ10 and Vκ-1 to 6). PCR is done in a volume of 50 μl using 0.5 μl cDNA, 1 unit Vent exo<sup>-</sup>-DNA-polymerase (New England Biolabs) and 0.5 μM of each primer under following conditions: 3 min 95 °C, 20x [30 sec 95 °C, 1 min 55 °C, 1 min 72 °C] 5 min 72 °C. The products of the first PCR are purified using the PCR purification Kit (Qiagen) and used as templates for as second PCR using a corresponding set of oligonucleotide primers of Fig. 9 to introduce restriction sites for cloning. The second PCR is carried out separately for each primer set according to the first PCR but using 1 min 57 °C for annealing. Products of the second PCR of the heavy chain, the lambda light chain and the kappa light chain are pooled and purified via PCR- purification Kit (Qiagen).

##### Cloning of the scFv phage display library

45 **[0041]** Heavy chain fragments are digested with NcoI and HindIII, light chain fragments with MluI and NotI (each New England Biolabs) according to the suppliers instructions and finally purified by gel extraction from 1% agarose gels using the Gel Extraction Kit (Qiagen). To create a sublibrary the heavy chains are cloned first into the phagedisplay vector pEXHAM1 (Figure 1) containing a stuffer scFv. Vector DNA is cut with NcoI and HindIII, purified via gel extraction and ligated separately with heavy chain fragments originating from different donors. Ligation is done in 20 μl volume using 50 ng vector, 9 ng heavy chain fragment and 1 unit T4 DNA-ligase (Roche) for three hours at room temperature. The ligation mixture is precipitated, resuspended in 10 μl water and mixed with 35 μl of electrocompetent *E.coli* XL1 blue cells (Stratagene) for electroporation according to the suppliers instructions. Transformed cells are plated on selective LB agarose plates containing 50 mM glucose, 100 μg/ml ampicillin and 20 μg/ml tetracyclin and incubated at 30 °C over night. The size of the sublibraries is in the range of  $1.5 \times 10^6$  to  $7.1 \times 10^7$  as determined by plating appropriate dilutions.

55 **[0042]** Bacterial clones are scraped from the plates and used for DNA-maxipreparation (Qiagen) to prepare the vector DNA for cloning of the complete libraries. Sublibrary DNA is cut with MluI and NotI, purified by gel extraction and ligated

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with lambda and kappa light chain fragments separately. Ligation is done in 20  $\mu$ l volume using 1  $\mu$ g vector DNA and a two fold molar excess of light chain DNA. After incubation with 1 unit T4 ligase (Roche) over night at 8 °C the ligation mixture is precipitated and redissolved in 2.5  $\mu$ l Tris 10mM, pH8.5. Of this 2  $\mu$ l are used for transformation of 50  $\mu$ l aliquots of electrocompetent XL1 blue cells. Cells are plated on selective agarplates and the number of transformants is determined by plating of appropriate dilutions as described above. The total size of all libraries generated from spleen and PBL RNA material is  $1.75 \times 10^9$ .

### Library rescue

**[0043]** For phage display of scFv's the library is inoculated in 250 ml aliquots of LB medium supplemented with 50 mM glucose, 100  $\mu$ g/ml ampicillin and 20  $\mu$ g/ml tetracyclin at a start OD600 of 0.025 ensuring that the number of cells exceeds the complexity by a factor of 10. Cells are incubated at 37 °C and 200 rpm until an OD600 of 0.2 and infected with M13K07 helperphages at a multiplicity of infection of 10. After one hour incubation at 37 °C cells are harvested by centrifugation, resuspended in 250 ml glucose free medium and incubated over night at 30 °C and 200 rpm. Phage are isolated by PEG precipitation (PEG6000 20%, NaCl 2.5M) and redissolved in phage dilution buffer (Tris 10 mM pH 7.5, NaCl 20 mM, EDTA 2 mM).

**[0044]** Screening the library for scFv's binding activated platelets Depletion of the library for scFv's binding non activated platelets:

5 ml of human, venous blood are collected in a S-Monovette (Sarstedt) containing 25  $\mu$ l prostaglandine E10 (10 mM) and centrifuged at 110g for 10 min. Of platelet rich plasma (upper phase) 1 ml is transferred into a fresh tube, mixed with 9 ml CGS-buffer (sodium citrate 10mM, dextrose 30mM, NaCl 120 mM) and centrifuged at 1000g for 10 min. The pellet is resuspended in 4 ml tyrode buffer (NaCl (150 mM), NaHCO<sub>3</sub> (12 mM), KCl, MgCl (2 mM each), glucose, BSA (1mg/ml each), pH 7.4) containing 2% skimmed milk powder and incubated with  $1.75 \times 10^{12}$  bacteriophages (1000x complexity) for 2 hours at room temperature. Platelets are centrifuged at 1000g for 10 min, the supernatand removed and stored at 4 °C

### Binding onto activated platelets:

**[0045]** 5 ml of human, venous blood are collected in a S-Monovette(Sarstedt) and centrifuged at 110g for 10 min. Of platelet rich plasma (upper phase) 1 ml is transferred in a fresh tube, mixed with 9 ml CGS-buffer and centrifuged at 1000g for 10 min. The pellet is resuspended with 4 ml depleted phage solution containing CaCl<sub>2</sub>, MgCl<sub>2</sub> (2 mM each), ADP (15  $\mu$ M) and incubated at roomtemperature for 2 hours. Platelets are washed twice by centrifugation (1000g, 10 min) and resuspended in 14 ml tyrode buffer.

### Elution:

**[0046]** For elution of binding phage the platelets are centrifuged (1000g, 10 min), resuspended in 1 ml glycine buffer (0.1 M, pH 2.2) and incubated for 10 min at room temperature. After centrifugation (1000g, 10 min) the supernatant is neutralized by addition of Tris (2 M, pH 8.0).

### Reinfection:

**[0047]** Eluted phages are mixed with 10 ml of logarithmic growing E. coli XL1 blue cells and incubated at 37 °C for 30 min. After centrifugation (10 min, 6000 g), cells are resuspended in 400  $\mu$ l LB<sub>GAT</sub> medium (LB medium containing 50 mM glucose, 100  $\mu$ g/ml ampicillin and 20  $\mu$ g/ml tetracyclin), plated on LB<sub>GAT</sub> agarplates and incubated over night at 37 °C.

### Packaging:

**[0048]** Colonies are scraped from agar plates using two times 5ml LB<sub>GAT</sub> medium and used for inoculation of 20 ml LB<sub>GAT</sub> medium at an OD600 of 0.1. Cells are incubated at 37 °C and 200 rpm for one hour and superinfected with app.  $1 \times 10^{10}$  M13K07 helperphages. After one hour at 37 °C cells are collected by centrifugation (5 min, 6000g) resuspended in LB medium supplemented with ampicillin (100 $\mu$ g/ml) and kanamycin (50  $\mu$ g/ml) and incubated over night at 30 °C and 200 rpm. Phages are collected by PEG precipitation and resuspended in 1 ml phage dilution buffer (as described for library rescue).

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Screening the library for scFv's binding recombinant GPIIb/IIIa on CHO-cells

Depletion of scfv's binding non activated GPIIb/IIIa:

5 **[0049]** Chinese hamster ovary cells (CHO) expressing non activated GPIIb/IIIa receptor (A5 cells; Peter et al., Blood, Vol. 9, 1998, pp. 3240-3249) are trypsinated, centrifuged (10 min, 140g) and resuspended at  $5 \times 10^6$  cells/ml in tyrode buffer. App.  $10^9$  packaged phage from the first round of selection are mixed with 4 ml cell suspension and incubated for one hour at room temperature. Cells are centrifuged for 20 min at 140g and the supernatant cleared again by centrifugation (20 min, 3200 g).

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Binding on activated GPIIb/IIIa:

15 **[0050]** CHO cells presenting active GPIIb/IIIa (C13 cells, Peter K and O'Toole TE, J Exp Med. 1995, 181(1): 315-326) are harvested by trypsination, centrifuged and washed once using 1 ml tyrode buffer.  $4 \times 10^6$  cells are incubated with 4 ml depleted phage solution for 30 min at room temperature.

Elution by antibody competition:

20 **[0051]** Cells are centrifuged for 10 min at 140g, resuspended in 50 ml tyrode buffer, three times centrifuged for 20 min at 700g and resuspended in 1ml tyrode buffer and finally resuspended in 200  $\mu$ l ReoPro (2mg/ml). After 20 min at room temperature cells are removed by 10 min centrifugation at 13000 rpm in a benchtop centrifuge.

Acidic elution:

25 **[0052]** Cells are centrifuged for 10 min at 140g, resuspended in 50 ml modified tyrode buffer (tyrode buffer pH 6 adjusted with Hepes, containing  $\text{CaCl}_2$ ,  $\text{MgCl}_2$  (2 mM each) and 1mg/ml BSA), twice centrifuged for 20 min at 700g and resuspended in 1 ml modified tyrode buffer and finally resuspended in 1 ml glycine (pH 2.2). After 15 min at room temperature the mixture is neutralized by addition of 100  $\mu$ l Tris (2 M, pH 8) and cleared by centrifugation at 13000rpm for 10 min in a benchtop centrifuge.

30 Reinfection and packaging: is done as described above.

**[0053]** Restriction endonuclease digestion analysis of selected clones DNA of clones from selection experiments are prepared using DNA spin columns following the recommendations of the manufacturer (Quiagen). DNA is digested with *Bst*NI (New England Biolabs) and analysed on a 1% agarose gel.

35 Preparation of periplasmic extracts

40 **[0054]** 5 ml of  $\text{LB}_{\text{GAT}}$  medium are inoculated with 250  $\mu$ l of an overnight culture and incubated at 37 °C and 180 rpm for 4 hours. Cells are harvested by centrifugation (5 min, 6000g) resuspended in 5 ml LB medium containing ampicillin (10  $\mu$ g/ml) and IPTG (100  $\mu$ M) and incubated at 28 °C and 180 rpm over night. Cells are again harvested by centrifugation and resuspended in 500  $\mu$ l shock solution (50 mM Tris HCl pH 8.0, 20% saccharose, 1 mM EDTA) and incubated at 8 °C for one hour. Cells are removed by centrifugation (10 min, 13000rpm benchtop centrifuge) and the supernatant dialysed two times 3 hours against PBS at 4°C.

FACS-analysis

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**[0055]** FACS-analysis is done using a FACSCalibur device (Becton Dickinson).

Analysis of activation specificity:

50 **[0056]** Complete citrate blood (S-Monovette, Sarstedt) is diluted 1/50 in 50  $\mu$ l tyrode buffer with or without ADP (20  $\mu$ M) and incubated for 20 min at room temperature with 10  $\mu$ l of periplasmic scFv extracts. As secondary antibody FITC labelled anti-His-antibody (Dianova) is added, incubated for 20 min and fixed with Cellfix (1x).

Analysis of fibrinogen competition:

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**[0057]** Complete citrate blood (S-Monovette, Sarstedt) is diluted 1/50 in 50  $\mu$ l tyrode buffer with or without ADP (20  $\mu$ M) and incubated for 20 min with FITC labeled anti-fibrinogen-antibody (WAK-Chemie Medical) in presence or absence of 20  $\mu$ l of periplasmic scFv extracts before fixation with Cellfix (1x, Becton Dickinson).

Results

Selection of GPIIb/IIIa binding scFv's

5 **[0058]** Human scFv phagedisplay libraries originating from spleen and PBL are screened for GPIIb/IIIa specific clones by selection on activated human platelets for one round. The library is depleted before on not activated platelets to remove not activation specific binders. The second and third round of selection is done on CHO cells expressing recombinant, activated GPIIa/IIIb receptor after depletion on cells presenting a not activated variant. Elution is done either by acid or by competition with ReoPro. After the third round of selection clones are randomly picked and analysed first for enrichment by *Bst*MI digestion and activation specific binding to thrombocytes (Table 1). One clone, MB9, is found to be enriched using acidic as well as competitive elution to 10 of 80 clones and 10 of 60 clones respectively. MB9 is also strongly activation specific in platelet binding and inhibits binding of fibrinogen to platelets as shown by FACS-analysis depicted in Figure 1. Therein, the following is depicted: Left histogram: demonstrates binding of MB9 scFv to activated (black) but not to unactivated (grey) human thrombocytes. Right histogram: Binding of fibrinogen to activated (black) but not to unactivated thrombocytes. Binding of fibrinogen to activated thrombocytes is inhibited in presence of MB9 scFv (filled bright grey curve).

15 **[0059]** Additionally MB9 competes with ReoPro for binding. Other enriched clones like MA1 showed also activation specific binding but failed in inhibition of fibrinogen binding or are not strongly specific for activated thrombocytes like MA3 or MB1.

20 **[0060]** The DNA sequence of clone MB9 is given in SEQ ID No. 1 (Fig. 2). Restriction endonuclease recognition sequences flanking heavy and light chains (*Nco*I, *Hind*III and *Mlu*I, *Not*I respectively) are indicated.

**[0061]** A clone encoding MB9 has been deposited under DSM 14491 (XL1blue(pEXHAM4/MP9)) on September 6, 2001 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig" under the Budapest Treaty.

Table 1: Characterisation of scFv clones enriched on activated GPIIb/IIIa.

Clone	elution done by	Enrichment Identical clones /analysed clones	Activation specific binding to human platelets	inhibition of fibrinogen binding	competition by ReoPro
MA1	Acid	20/80	++	-	-
MA2	Acid	10/80	++	++	+
MA3	Acid	24/80	+	-	-
MB1	Competition	21/60	+	+	+
MB9 Identical to MA2	Competition	10/60	++	++	+

++: strongly positive; +: positive; -: negative

**Example 2: Production of the synthetic human framework based scFv antibody fragment**

Origin of human scFv master frameworks

45 **[0062]** For the generation of a synthetic library by randomization of the CDR3 region of the heavy chain two human master frameworks (C9 and E4, Figure 3) are chosen because of their excellent production characteristics in *E. coli* cells. Both scFv's originate from a large human phage display antibody library (Little, M., et al., J. Immunol. Methods 1999, 231: 3-9) and specific for hepatitis B virus antigen (C9) and estradiol (E4) respectively.

Vector construction for the synthetic scFv library

55 **[0063]** C9 and E4 scFv's are cloned in pEXHAM1 vector DNA replacing the stuffer scFv by standard recombinant cloning techniques using *Nco*I and *Not*I cloning sites. To prepare a vector allowing the randomization of CDR3 of the heavy chain without changes of the original sequence this region is replaced by a stuffer DNA fragment containing restriction enzyme recognition sites of the type IIS enzyme

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*Bbsl* (*Bpil*). Standard PCR reactions are set up using the oligonucleotide primers as shown in Fig. 4 to generate DNA fragments of the scFv regions 3' and 5' of the heavy chain CDR3 containing unique *Bpil* cloning sites as outlined in Fig. 5, which is a schematic representation of annealing positions of oligonucleotides used for the construction of pEXHAM4/C9 and pEXHAM4/E4. Genes of the scFv's C9 and E4 cloned in pEXHAM1 are shown as boxes. Black painted areas represent CDR regions; Oligonucleotides are represented by arrows and identified by numbers (cp. sequence definitions). *Bpil* restriction endonuclease recognition sites are indicated.

[0064] The stuffer DNA fragment is generated directly by hybridisation of synthetic oligonucleotides. DNA-fragments are cut with *Bpil* and cloned in *Bpil* digested pEXHAM1 vector DNA to generate pEXHAM4/C9 and pEXHAM4/E4 (Figs. 6, 11 and 12).

[0065] Direct use of pEXHAM4 vector DNA for cloning via *Bbsl* necessitates the purification of two vector fragments, 3.8 and 0.5 kb in size. To avoid this both *Bbsl* restriction sites outside the scFv sequence are removed in several steps without changing the protein sequence using mismatched oligonucleotides as primers for PCR or directly hybridised synthetic oligonucleotides to replace *Bbsl* containing DNA-fragments by cloning via neighboring restriction sites. The final constructs is named pEXHAM7/C9 and pEXHAM7/E4 (Figs. 7, 13 and 14) respectively.

Generation of the synthetic, human framework based scFv library

[0066] To generate a library synthetic oligonucleotides encoding four to seven random aminoacids by NNK codons (VHCDR3\_3.4/cut until VHCDR3\_3.7/cut; 1  $\mu$ M each) are filled in separately using oligonucleotides VHCDR3\_for/cut and VHCDR3\_back/cut (0.2  $\mu$ M) (Fig. 4) with 1 unit Vent exo<sup>-</sup> DNA-polymerase (New England Biolabs) under following PCR conditions: 2 min 94 °C, 5x [1 min 94 °C, 1 min 40 °C, 1 min 72 °C] 10 min 72 °C in 100  $\mu$ l Volume. PCR-products are purified using PCR purification Kit (Qiagen). 2/3 of the material is cut with 100 units *Bbsl* for 6 hours and again purified via the mentioned kit. In case of VHCDR3\_3.4/cut and VHCDR3\_3.5/cut the vector DNA pEXHAM4/C9 and pEXHAM4/E4 is cut with *Bbsl* (1 unit/ $\mu$ g in 6 hours) and both vector fragments (3.8 and 0.5 kb) are purified via gel elution from an 1 % agarose gel (Gel Extraction Kit, Quiagen). For VHCDR3\_3.6/cut and VHCDR3\_3.7/cut pEXHAM6/C9 and pEXHAM6/E4 are used, therefore only one vector fragment had to be purified. Ligation is done in all cases at an equimolar ratio of all fragments. Afterwards the ligation mixture is precipitated, redissolved in Tris 10mM, pH8.5 and used for transformation of XL1 blue cells essentially as described for example 1.

[0067] In addition to synthetic randomized DNA-fragments, CDR3 of the heavy chain is also replaced by natural CDR3 sequences amplified from the products of the first PCR of the natural library (see example 1) to focus on functional, *in vivo* used sequences for this region. Oligonucleotides used and described in Fig. 4 are designed to cover most of the human heavy chain CDR3 regions without modifying C9 or E4 framework sequences. PCR is done separately for each human VH PCR template using 1 unit Vent exo<sup>-</sup>-DNA polymerase (New England Biolabs) and 0.2  $\mu$ M primer in a volume of 100  $\mu$ l under following conditions: 2 min 94°C, 30x [1 min 95 °C, 1 min 50 °C, 1 min 72 °C] 10 min 72 °C. Oligo nucleotides #42, #43 and #44 are used as an equimolar mixture. PCR products are purified via PCR purification kit and material originating from spleen or PBL respectively is pooled. Restriction with *Bbsl*, ligation with pEXHAM6/C9 and pEXHAM6/E4 respectively and transformation is done as described above.

[0068] The size of the whole synthetic library (synthetic and natural CDR3's cloned in C9 or E4 frameworks) in this example is  $7.5 \times 10^8$  clones.

Library rescue

[0069] Packaging of synthetic libraries is done as described for the natural library (example 1).

Screening of the synthetic library

[0070] Screening of the synthetic library is done exactly as described for the natural library (example 1) starting with  $7.5 \times 10^{11}$  bacteriophages (1000x complexity).

Results

[0071] The synthetic library derived from human scFv frameworks (C9 and E4) is screened for GPIIb/IIIa specific clones exactly as described in example 1. After the third round of selection clones are randomly picked and the DNA sequence of the VH-CDR3 regions was determined (c.f. Table 2)

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Table 2: Analysis of the DNA-Sequence of VH-CDR3 of eleven GPIIb/IIIa selected clones from the synthetic library

clone	translation of VH-CDR3 DNA	no of identical clones	oligonucleotide used for CDR3
SA1	CAR RYRVG FDY	1	VHCDR3_ 3.5/cut
SA2	CAR GATYTSRSDVPDQTS FDY	2	VHCDR3_ ev2/for/cut
SA3	CAR DDLAYCRGDCSGRFA FDI	2	VHCDR3_ ev2/for/cut
SA4	CAR RFSISRA FDY	1	VHCDR3_ 3.7/cut
SA6	CAR RWGKARS FDY	1	VHCDR3_ 3.7/cut
SA8	CAK ELEAYCRGDCYPPYYG MDV	1	VHCDR3_ ev3/for/cut
SA10	CAR DLFRGRGDYGDYD MDV	1	VHCDR3_ ev2/for/cut
SA11	CAR TYYYDSRTDRRPPHA FDI	1	VHCDR3_ ev3/for/cut

**[0072]** All of the clones use the E4 framework sequence. Three of the eleven analysed clones encode the amino acid sequence RGD (also present in fibrogen) within CDR3 (SA3, SA8 and SA10). In clones SA3 and SA8 the RGD motive is directly flanked by two cysteine residues that might stabilize the loop by disulfide bridges. Clone SA3 was found twice under eleven analysed clones and, therefore, has probably enriched by the screening procedure. The same is true for clone SA11. These scFv clones are similar to antibodies like PAC-1 that contain RGD-like sequences and inhibit fibrogen binding by blocking the activated receptor (Shatill et al., 1985). Only SA8, Sa10 and SA11 showed an activation specific binding to thrombocytes in the presence of fibrinogen (c.f. Fig. 10).

**[0073]** The selected clones probably interact exactly with the fibrinogen binding site of the GPIIb/IIa receptor but with an affinity similar or lower than fibrinogen. The affinity has been enhanced by mutation within the VH and/or the VL-domain of the scFv antibody fragment or the exchange of the whole VL domain ("chain shuffling").

SEQUENCE LISTING

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<220>  
<223> Description of the Artificial Sequence: Plasmid

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<220>  
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<210> 56

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<210> 57

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<223> Description of Artificial Sequence: Oligonucleotide

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<210> 71  
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<210> 72  
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<210> 73  
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<400> 73  
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<210> 74  
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<223> Description of Artificial Sequence: oligonucleotide

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tggacgccca tggcgcaggt ccagctggta cagtct 36

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<210> 75

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<211> 36

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<210> 78

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<210> 79

<211> 36

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<220>

<223> Description of Artificial Sequence: oligonucleotide

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<210> 92

<211> 36

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<210> 95

<211> 36

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<210> 97  
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<210> 98  
<211> 36  
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<210> 101  
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Oligonucleotide

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<210> 114  
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<220>  
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<400> 116

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55 <400> 121  
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<210> 122

<211> 36  
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<220>  
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<210> 123  
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<210> 124  
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<220>  
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<210> 126  
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<220>

<223> Description of Artificial Sequence:peptide fragment

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Glu Leu Glu Ala Tyr Cys Arg Gly Asp Cys Tyr Pro Pro Tyr Tyr Gly  
1 5 10 15

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<220>

<223> Description of Artificial Sequence:peptide fragment

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<210> 129

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<220>

<223> Description of Artificial Sequence:peptide fragment

<400> 129

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Cys Ala Arg Gly Ala Thr Tyr Thr Ser Arg Ser Asp Val Pro Asp Gln  
1 5 10 15

Thr Ser Phe Asp Tyr  
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40

<210> 130

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<213> Artificial Sequence

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<220>

<223> Description of Artificial Sequence:peptide fragment

<400> 130

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Cys Ala Arg Asp Asp Leu Ala Tyr Cys Arg Gly Asp Cys Ser Gly Arg  
1 5 10 15

Phe Ala Phe Asp Ile  
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<210> 131

<211> 13

<212> PRT

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<213> Artificial Sequence

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<223> Description of Artificial Sequence:peptide fragment

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Tyr Tyr Gly Met Asp Val  
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<210> 134

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<211> 19

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<220>

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<223> Description of Artificial Sequence:peptide fragment

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Met Asp Val

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<210> 135

<211> 21

10 <212> PRT

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<223> Description of Artificial Sequence:peptide fragment

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His Ala Phe Asp Ile  
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<210> 136

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<210> 137

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<210> 138

<211> 36

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<213> Artificial Sequence

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<210> 142  
<211> 75  
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45 **ggaaccctgg tcacc 75**

<210> 143  
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**caggggaacc tggtcacc 78**

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20 <210> 145  
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25 <220>  
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30 **gaggacacgg ctgtatatta ctgtgcgara nnknnknnkn nknnknnknn ktttgastac 60**  
**tggggccagg gaaccctggt cacc 84**

**Claims**

- 35
1. Antibody or derivative thereof of human origin for inhibiting platelet aggregation, **characterised in that** it is effective by substantially exclusive binding to the activated state of platelet integrin receptor GPIIb/IIIa.
  2. Antibody according to claim 1, **characterised in that** the antibody derivative is a fragment of an immunoglobulin comprising variable domains of its light and heavy chains.
  - 40 3. Antibody according to claim 2, **characterised in that** the fragment is a single chain antibody fragment (scFv) or Fab.
  4. Antibody according to claim 2 or 3, **characterised in that** the fragment is a recombinant construct of a single chain antibody fragment (scFv).
  - 45 5. Antibody according to claim 2 or 3, **characterised in that** the single chain antibody fragment is derived from an IgM or IgG antibody.
  - 50 6. Antibody according to claim 3 or 4, **characterised in that** the fragment comprises the amino acid sequence of Fig. 2 or an amino acid sequence that is at least 60% homologous thereto
  7. Antibody according to any of claims 1-6, **characterised in that** it is a bivalent or multivalent antibody construct comprising the variable domain of an antibody specific to bind to the activated state of platelet integrin receptor GPIIb/IIIa.
  - 55 8. A process for identifying and/or isolating antibodies for inhibiting platelet aggregation by binding to an activated integrin receptor GPIIb/IIIa of blood thrombocytes, comprising the following steps:

- providing a library of nucleic acids containing a sequence for a single chain antibody fragment containing a heavy and a light variable domain; and
  - introducing at least one randomised nucleotide sequence into the CDR region of said single chain antibody fragment.
- 5
- producing a phage library from said nucleic acids library;
  - successively reacting said phage library with nonactive thrombocytes, active thrombocytes, other cells expressing nonactive integrin receptor GPIIb/IIIa molecules, and other cells expressing active integrin receptor GPIIb/IIIa molecules; and
  - eluting phages bound to said thrombocytes or other cells expressing active integrin receptor molecules.
- 10

9. DNA sequence coding for the antibody according to any of claims 1-7.

10. Expression vector containing the DNA sequence according to claim 9.

15 11. Cell line containing the DNA sequence according to claim 9 or the expression vector according to claim 10.

12. Pharmaceutical composition containing the antibody according to any of claim 1-7, the DNA sequence according to claim 9 or the expression vector according to claim 10.

20 13. Use of the antibody according to any of claim 1-7, the DNA sequence according to claim 9 or the expression vector according to claim 10 for preparing a pharmaceutical composition for blocking the platelet integrin receptor on thrombocytes.

25 14. Use of the antibody according to any of claim 1-7, the DNA sequence according to claim 9 or the expression vector according to claim 10 as a diagnostic for determining the number of activated thrombocytes.

#### Patentansprüche

30 1. Antikörper oder dessen Derivat menschlichen Ursprungs zum Hemmen einer Blutplättchenaggregation, **dadurch gekennzeichnet, dass er dadurch wirksam ist, dass er im Wesentlichen ausschließlich an den aktivierten Zustand eines Blutplättchenintegrinrezeptors GPIIb/IIIa bindet.**

35 2. Antikörper nach Anspruch 1, **dadurch gekennzeichnet, dass** das Antikörperderivat ein Fragment eines Immunglobulins ist, das variable Domänen seiner leichten und schweren Kette umfasst.

3. Antikörper nach Anspruch 2, **dadurch gekennzeichnet, dass** das Fragment ein einzelkettiges Antikörperfragment (scFv) oder Fab ist.

40 4. Antikörper nach Anspruch 2 oder 3, **dadurch gekennzeichnet, dass** das Fragment ein rekombinantes Konstrukt eines einzelkettigen Antikörperfragments (scFv) ist.

45 5. Antikörper nach Anspruch 2 oder 3, **dadurch gekennzeichnet, dass** das einzelkettige Antikörperfragment von einem IgM oder IgG Antikörper stammt.

6. Antikörper nach Anspruch 3 oder 4, **dadurch gekennzeichnet, dass** das Fragment die Aminosäuresequenz von Fig. 2 oder eine Aminosäuresequenz umfasst, die mindestens 60 % homolog dazu ist.

50 7. Antikörper nach einem der Ansprüche 1 bis 6, **dadurch gekennzeichnet, dass** er ein bivalentes oder multivalentes Antikörperkonstrukt ist, das die variable Domäne eines Antikörpers umfasst, der dahingehend spezifisch ist, dass er an den aktivierten Zustand des Blutplättchenintegrinrezeptors GPIIb/IIIa bindet.

55 8. Verfahren zum Identifizieren und/oder Isolieren von Antikörpern zum Hemmen einer Blutplättchenaggregation durch Bindung an einen aktivierten Integrinrezeptor GPIIb/IIIa von Blutthrombozyten, umfassend die folgenden Schritte:

- Erstellen einer Bibliothek von Nukleinsäuren, die eine Sequenz für ein einzelkettiges Antikörperfragment enthalten, das eine schwere und eine leichte variable Domäne umfasst; und
- Einführen von mindestens einer randomisierten Nukleotidsequenz in die CDR Region des einzelkettigen

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Antikörperfragments;

- Erzeugen einer Phagenbibliothek aus der Nukleotidsäurebibliothek;
- aufeinander folgende Reaktion der Phagenbibliothek mit nicht aktiven Thrombozyten, aktiven Thrombozyten, anderen Zellen, die nicht aktive Integrinrezeptor-GPIIb/IIIa-Moleküle exprimieren, und anderen Zellen, die aktive Integrinrezeptor-GPIIb/IIIa-Moleküle exprimieren; und
- Elution von Phagen, die an die Thrombozyten oder andere Zellen, die aktive Integrinrezeptormoleküle exprimieren, binden.

9. DNA Sequenz, die für den Antikörper nach einem der Ansprüche 1 bis 7 kodiert.

10. Expressionsvektor, enthaltend die DNA Sequenz nach Anspruch 9.

11. Zelllinie, enthaltend die DNA Sequenz nach Anspruch 9 oder den Expressionsvektor nach Anspruch 10.

12. Pharmazeutische Zusammensetzung, enthaltend den Antikörper nach einem der Ansprüche 1 bis 7, die DNA Sequenz nach Anspruch 9 oder den Expressionsvektor nach Anspruch 10.

13. Verwendung des Antikörpers nach einem der Ansprüche 1 bis 7, die DNA Sequenz nach Anspruch 9 oder den Expressionsvektor nach Anspruch 10 zur Herstellung einer pharmazeutischen Zusammensetzung zum Blockieren des Blutplättchenintegrinrezeptors auf Thrombozyten.

14. Verwendung des Antikörpers nach einem der Ansprüche 1 bis 7, der DNA Sequenz nach Anspruch 9 oder des Expressionsvektors nach Anspruch 10 als diagnostisches Mittel zum Bestimmen der Zahl von aktivierten Thrombozyten.

### Revendications

1. Anticorps ou son dérivé d'origine humaine pour l'inhibition de l'agrégation des plaquettes, **caractérisé en ce qu'il** est efficace en se liant essentiellement exclusivement à l'état activé du récepteur GPIIb/IIIa à l'intégrine des plaquettes.

2. Anticorps selon la revendication 1, **caractérisé en ce que** le dérivé d'anticorps est un fragment d'immunoglobuline comprenant des domaines variables de ses chaînes lourdes et légères.

3. Anticorps selon la revendication 2, **caractérisé en ce que** le fragment est un fragment d'anticorps simple chaîne (scFv) ou Fab.

4. Anticorps selon la revendication 2 ou 3, **caractérisé en ce que** le fragment est une construction recombinante d'un fragment d'anticorps simple chaîne (scFv).

5. Anticorps selon la revendication 2 ou 3, **caractérisé en ce que** le fragment d'anticorps simple chaîne est dérivé d'un anticorps IgM ou IgG.

6. Anticorps selon la revendication 3 ou 4, **caractérisé en ce que** le fragment comprend la séquence d'acides aminés de la figure 2 ou une séquence d'acides aminés qui lui est homologue à au moins 60%.

7. Anticorps selon l'une quelconque des revendications 1 à 6, **caractérisé en ce qu'il** est une construction d'anticorps bivalent ou multivalent comprenant le domaine variable d'un anticorps spécifique à se lier à l'état activé du récepteur GPIIb/IIIa à l'intégrine des plaquettes.

8. Procédé d'identification et/ou d'isolation d'anticorps pour l'inhibition de l'agrégation des plaquettes par liaison avec un récepteur GPIIb/IIIa à l'intégrine activé, des thrombocytes sanguins, comprenant les étapes suivantes :

- fournir une bibliothèque d'acides nucléiques contenant une séquence pour un fragment d'anticorps simple brin contenant un domaine variable lourd et un domaine variable léger, et
- introduire au moins une séquence nucléotidique randomisée dans la région CDR dudit fragment d'anticorps simple chaîne,

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- produire une bibliothèque de phages à partir de ladite bibliothèque d'acides nucléiques,
- faire réagir successivement ladite bibliothèque de phages avec des thrombocytes non actifs, des thrombocytes actifs, d'autres cellules exprimant des molécules de récepteur GPIIb/IIIa à l'intégrine non actifs, et d'autres cellules exprimant des molécules de récepteur GPIIb/IIIa à l'intégrine actifs, et
- éluer les phages liés aux dits thrombocytes ou aux autres cellules exprimant les molécules de récepteur à l'intégrine actif.

5

9. Séquence d'ADN encodant l'anticorps selon l'une quelconque des revendications 1 à 7.

10

10. Vecteur d'expression contenant la séquence d'ADN selon la revendication 9.

11. Lignée cellulaire contenant la séquence d'ADN selon la revendication 9 ou le vecteur d'expression selon la revendication 10.

15

12. Composition pharmaceutique contenant l'anticorps selon l'une quelconque des revendications 1 à 7, la séquence d'ADN selon la revendication 9 ou le vecteur d'expression selon la revendication 10.

20

13. Utilisation de l'anticorps selon l'une quelconque des revendications 1 à 7, de la séquence d'ADN selon la revendication 9 ou du vecteur d'expression selon la revendication 10 pour préparer une composition pharmaceutique destinée à bloquer le récepteur à l'intégrine des plaquettes sur les thrombocytes.

25

14. Utilisation de l'anticorps selon l'une quelconque des revendications 1 à 7, de la séquence d'ADN selon la revendication 9 ou du vecteur d'expression selon la revendication 10 comme diagnostic pour déterminer le nombre de thrombocytes activés.

30

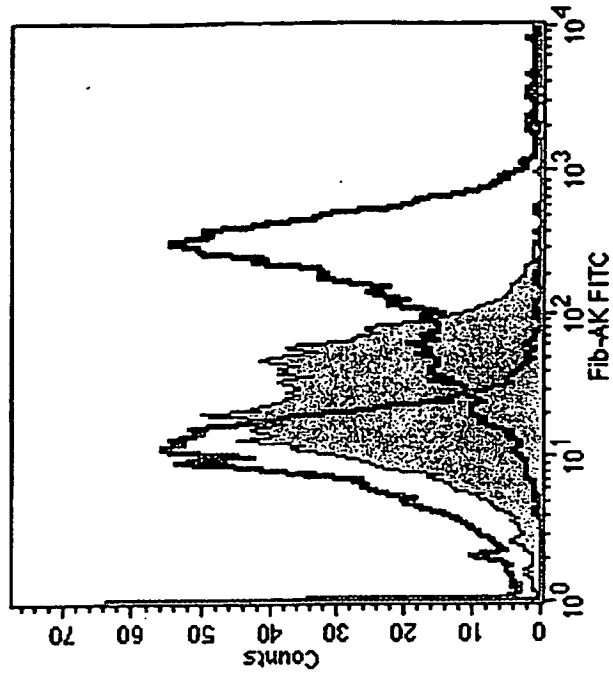
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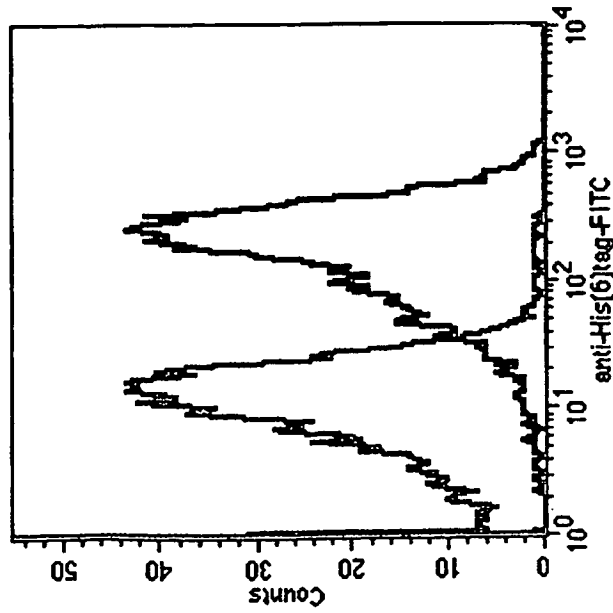
45

50

55



Key	Name	Parameter	Gate
█	FG+2.036	FL1-H	G1
—	FG-.034	FL1-H	G1
—	FG+.033	FL1-H	G1



Key	Name	Parameter	Gate
█	MA2-.004	FL1-H	G1
—	MA2+.005	FL1-H	G1

Fig. 1

SEQ No. 1

*NcoI*  
 1 ccatggcgga agtgcagctg gtgcagtctg gagctgaggt gaataagcct ggggcctcag  
 61 tgaaggctc ctgcaaggct tctggataca ccttcaccgg ctactatatg cactgggtgc  
 121 gacaggcccc tggacaaggg cttgagtgga tgggatggat caaccctaac agtggtgga  
 181 caaactatgc acagaagttt cagggtctggg tcaccatgac cagggacacg tccatcagca  
 241 ccgcctacat ggagctgagc aggctgagat ctgacgacac ggccgtgtat tactgtgcga  
 301 gaggccgtgc tttgtataac cggaacgacc ggtcccccaa ctggttcgac cctggggcc  
*HindIII*  
 361 agggaaccct ggtcacctgc tcctcaggga gtgcatccgc cccaaccctt aagcttgaag  
*MluI*  
 421 aaggtgaatt ttcagaagca cgcgtacagg ctgtgctgac tcagccgcc tcggtgtcag  
 481 tggccccagg acagacggcc aggattacct gtgggggaaa caacattgga agtaaaagtg  
 541 tgcagtggta ccagcagaag ccaggccagg cccctgtgct ggtcgtctat gatgatagcg  
 601 accggccctc agggatccct gagegattct ctggctccaa ctctgggaac atggccaccc  
 661 tgaccatcag cagggtcgaa gccggggatg aggccgacta ttactgtcag gtgtgggata  
 721 gtagtagtga tcatgtgga ttcggcggag ggaccaagct gaccgtccta ggtcagccca  
*NoI*  
 781 aggetgcccc ctcggtcact ctgttcccgc cgtccgggc cgc

Fig. 2a

Translation of MB9

1	MAEVQLVQSG	AEVNKPGASV	KVSCKASGYT	FTGYMHWVR	QAPGQGLEWM
51	GWINPNSGGT	NYAQKFGWV	TMTRDTSIST	AYMELSRRLRS	DDTAVYYCAR
101	GRALYNRNRD	SPNWFDPWGQ	GTLVTVSSGS	ASAPTLKLEE	GEFSEARVQA
151	VLTQPPSVSV	APGQTARITC	GGNNIGSKSV	QWYQKPGQA	PVLVVYDSD
201	RPSGIPERFS	GSNSGNMATL	TISRVEAGDE	ADYICQWDS	SSDHVVFSGG
251	TKLTVLQQPK	AAPSVTLFPP	SAAAGSHHHH	HH*	

Fig. 2b

C9 scFv: (Seq. No. 2)

*NcoI*

1 ccatggcgca ggtacagctg caggagtctg ggggaggcgt ggtccagcct gggaggtccc  
61 tgagactctc ctgtgcagcc tctggattct ccttcagtaa ttatggcata cactgggtcc  
121 gccaggctcc aggcaagggg ctggagtggg tggcacttat atcatatgat gaaataaga  
181 aattctatgc agactccgtg aaggccgat tgcctatctc cagagacact tctaagaata  
241 cgggtgatct gcaaatgacc agcctgagac ctgaggacac ggctgtatat tactgtgcga  
301 aatctggggg tattgccttg tactgggggg aatttgacta ctggggccag ggaaccctgg

*HindIII*

361 tcaccgtctc ctcagcctcc accaagggcc caaagcttga agaaggtgaa ttttcagaag

*MluI*

421 cacgcgtatc ctatgaactg actcagccac cctcgggtgc agtggcccca ggacagacgg  
481 ccatgattac ctgtggggga aacaacattg gaagtacaac cgtgcactgg tatcagcaga  
541 agccaggcca ggcccctgtg ctggtcgtct atgatgataa cgagcgaccc tcagggatcc  
601 ctgagcgatt ctctggctcc aactctggga gcacggccac cctgaccatc aacagggctc  
661 aagccgggga tgaggccgac tattattgtc aagtgtggga tagtggtagt gatcatgtgg  
721 tattcggcgg agggacgaag ctgaccgtcc taggtcagcc caaggctgcc ccctcggta

*NotI*

781 ctctgttccc gccctcctct gcggccgc

E4 scFv: (Seq. No. 3)

*NcoI*

1 ccatggcgca ggtgcagctg caggagtctg ggggaggcct ggtacagcct ggggggtccc  
61 tgagactctc ctgtgcagcc tctggattca tgtttagcag gtatgccatg agctgggtcc  
121 gccaggctcc aggaagggg ccagagtggg tctcaggtat tagtggtagt ggtggtagta  
181 catactacgc agactccgtg aaggccggt tcaccgtctc cagagacaat tccaagaaca  
241 cgctgtatct gcaaatgaac agcctgagag ccgaggacac ggccgtatat tactgtgcga  
301 aagatctggg ctactatggt tcggggagcc aacccttga gtactggggc cagggaaact

*HindIII*

361 tggtcaccgt ctcctcaggg agtgcacccg ccccaagct tgaagaaggt gaattttcag

*MluI*

421 aagcacgcgt atctgaactg actcaggacc ctgctgtgtc tgtggccttg ggacagacag  
481 tcaggatcac atgccaagga gacagcctca gaaactttta tgcaagctgg taccagcaga  
541 agccaggaca ggcccctact cttgtcatct atggtttaag taaaaggccc tcagggatcc  
601 cagaccgatt ctctgcctcc agctcaggaa acacagcttc cttgaccatc actggggctc  
661 aggcggaaga tgaggctgac tattactgta actcccggga cagaagtggg aatcatgtaa  
721 atgtgctatt cggcggaggg accaagctga ccgtcctacg tcagcccaag gctgccccct

*NotI*

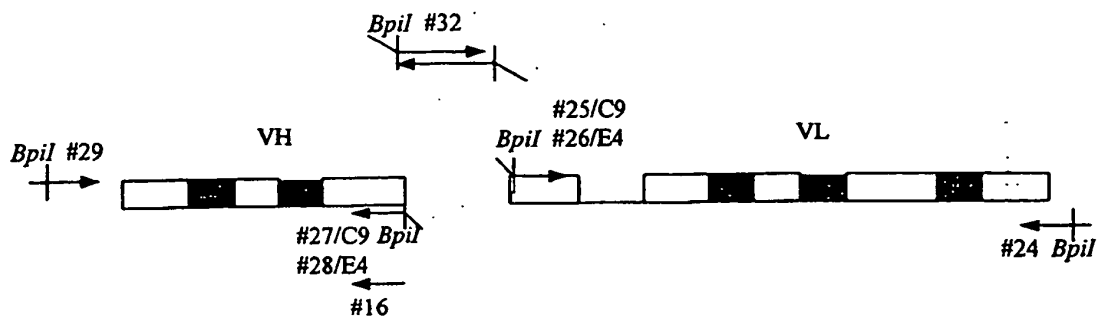
781 cgtcactct gttcccgcc tcttctg cgccgc

Figure 3: DNA sequence of C9 and E4 scFv masterframeworks  
Restriction endonuclease recognition sequences flanking heavy and light chains (*NcoI*, *HindIII* and *MluI*, *NotI* respectively) are indicated.

Oligonucleotides used for the construction of the human scFv based synthetic antibody library. *Bbs*I restriction enzyme recognition sites are indicated in bold style cut sites are underlined.

vector construction	library construction
<p>C9/VHCDR1.2/back/cut (#27): TAC TAC GAA GAC GTG <b>TCC</b> TCA GGT CTC AGG CTG GTC</p> <p>E4/VHCDR1.2/back/cut (#28): TAC TAC GAA GAC GTG <b>TCC</b> TCG GCT CTC AGG CTG TTC</p> <p>VH/for/cut (#29): AAT GCA GGT ATC ACG AGG <b>CCC</b> <b>TTT</b> CGT CTT C</p> <p>VL/back/cut (#24): CAG CTC TGA TAT CTT TGG ATC <b>CGT</b> <b>TTA</b> GGT CTT CTT CTG</p> <p>C9/VL/for/cut (#25): TAC TAC GAA GAC TGG <b>TCA</b> CCG TCT CCT CAG CCT CCA</p> <p>E4/VL/for/cut (#26): TAC TAC GAA GAC TGG <b>TCA</b> CCG TCT CCT CAG GGA GTG</p> <p>VHCDR3/stuff/for (#32): <b>GGA</b> <b>CAC</b> GTC TTC AGC GCT GAG CTC GAA GAC TG</p> <p>VHCDR3/stuff/back (#33): <b>TGA</b> <b>CCA</b> GTC TTC GAG CTC AGC GCT GAA GAC GT</p>	<p>VHCDR3_3.4/cut: GAG GAC ACG GCT GTA TAT TAC TGT GCG ARA (NNK)<sup>4</sup> TTT GAS TAC TGG GGC CAG GGA ACC CTG GTC ACC</p> <p>VHCDR3_3.5/cut: GAG GAC ACG GCT GTA TAT TAC TGT GCG ARA (NNK)<sup>5</sup> TTT GAS TAC TGG GGC CAG GGA ACC CTG GTC ACC</p> <p>VHCDR3_3.6/cut: GAG GAC ACG GCT GTA TAT TAC TGT GCG ARA (NNK)<sup>6</sup> TTT GAS TAC TGG GGC CAG GGA ACC CTG GTC ACC</p> <p>VHCDR3_3.7/cut: GAG GAC ACG GCT GTA TAT TAC TGT GCG ARA (NNK)<sup>7</sup> TTT GAS TAC TGG GGC CAG GGA ACC CTG GTC ACC</p> <p>VHCDR3_for/cut: AGC CTG GAA GAC GAG <b>GAC</b> ACG GCT GTA TAT TAC TGT GCG A</p> <p>VHCDR3_back/cut: GGC TGA GAA GAC GGT <b>GAC</b> CAG GGT TCC CTG GCC CCA GTA</p> <p>VHCDR3_ev1/for/cut (#42): AGC CTG GAA GAC GAG <b>GAC</b> ACG GCY GTG TAT TAC TGT</p> <p>VHCDR3_ev2/for/cut (#43): AGC CTG GAA GAC GAG <b>GAC</b> ACW GCC GTG TAT TAC TGT</p> <p>VHCDR3_ev3/for/cut (#44): AGC CTG GAA GAC GAG <b>GAC</b> ACG GCC GTA TAT TAC TGT</p> <p>VHCDR3_ev/back/cut (#45): GGC TGA GAA GAC GGT <b>GAC</b> CAG GGT KCC CTG GCC CCA</p>

Fig. 4



Schematic representation of annealing positions of oligonucleotides used for the construction of pEXHAM4/C9 and pEXHAM4/E4. Genes of the scFv's C9 and E4 cloned in pEXHAM1 are shown as boxes. Black painted areas represent CDR regions; Oligonucleotides are represented by arrows and identified by numbers (Fig. 4). *BpiI* restriction endonuclease recognition sites are indicated.

Fig. 5

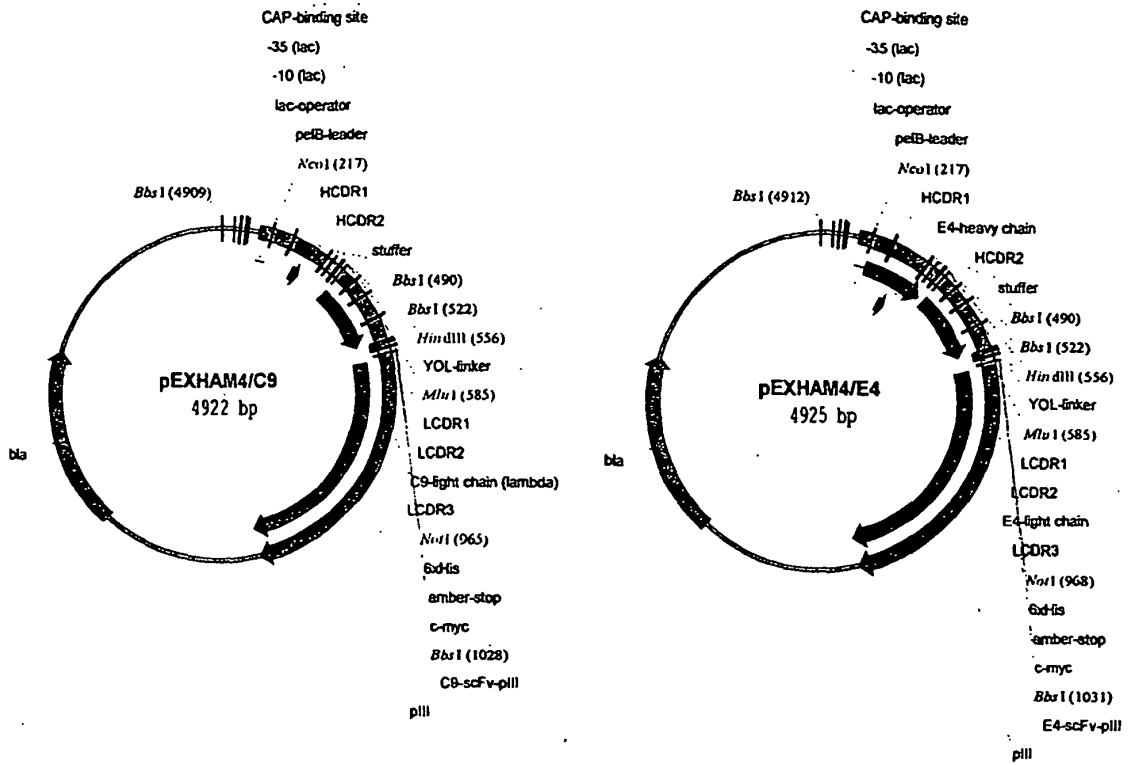


Figure 6: Vectormaps of pEXHAM4/C9 and pEXHAM4/E4

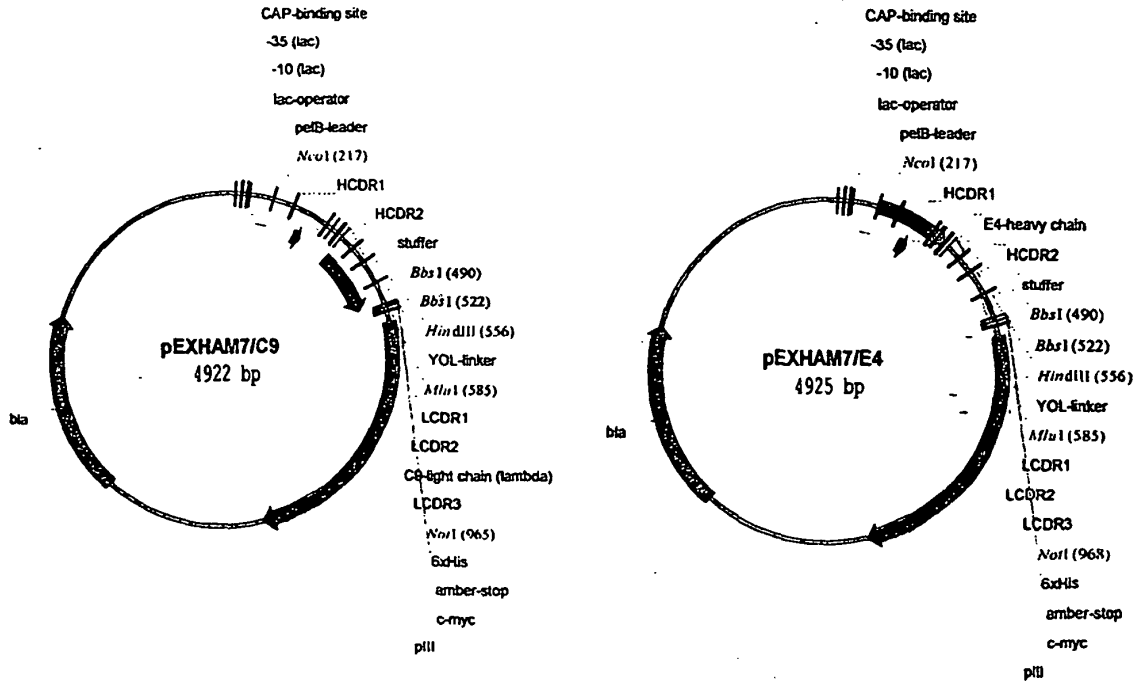


Figure 7: Vectormaps of pEXHAM7/C9 and pEXHAM7/E4

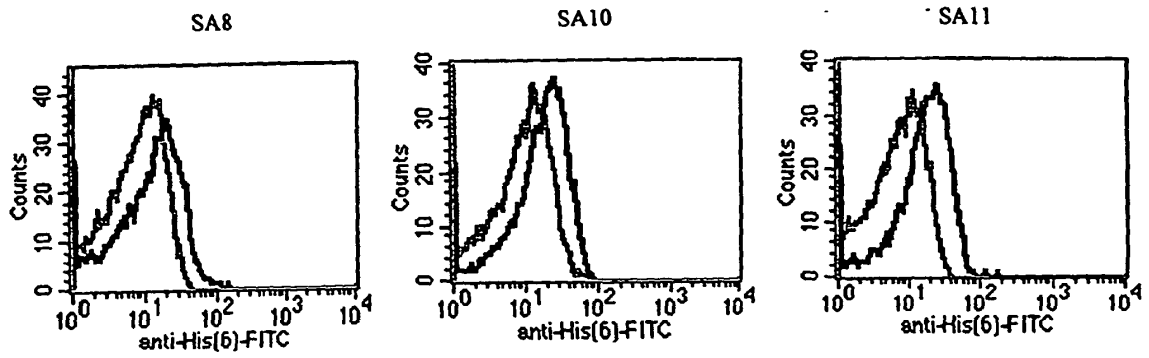
Oligonucleotides used as primers in 1. PCR for amplification of human heavy and light chain variable regions	
heavy chain primer	light chain primer
VH-1a. CAG GTG CAG CTG GTG CAG TCT 22	VA-1a. CAG TCT GTG CTG ACG CAG CCA 34
VH-1b. CAG GTC CAG CTT GTG CAG TCT	VA-1b. CAG TCT GTG CTG ACG CAG CCG
VH-1c. CAG GTC CAG CTG GTA CAG TCT	VA-2. CAG TCT GCC CTG ACT CAG CCT
VH-1d. GAG GTC CAG CTG GTA CAG TCT	VA-3a. TCC TAT GAG CTG ACA CAG CCA
VH-1e. CAG ATG CAG CTG GTA CAG TCT	VA-3b. TCC TCT GAG CTG ACA CAG GAC
VH-2a. CAG ATC ACC TTG AAG GAG TCT	VA-3c. TCC TAT GTG CTG ACA CAG CCA
VH-2b. CAG GTC ACC TTG AAG GAG TCT	VA-3d. TCC TAT GAG CTG ACA CAG CTA
VH-3a. GAA GTG CAG CTG GTG GAG TCT	VA-3e. TCC TAT GAG CTG ATG CAG CCA
VH-3b. CAG GTG CAG CTG GTG GAG TCT	VA-4a. CTG CCT GTG CTG ACT CAG CCC
VH-3c. GAG GTG CAG CTG TTG GAG TCT	VA-4b. CAG CCT GTG CTG ACT CAA TCA
VH-4a. CAG GTG CAG CTG CAG GAG TCG	VA-4c. CAG CTT GTG CTG ACT CAA TCG
VH-4b. CAG CTG CAG CTG CAG GAG TCG	VA-5a. CAG CCT GTG CTG ACT CAG CCA
VH-4c. CAG GTG CAG CTA CAG CAG TGG	VA-5b. CAG GCT GTG CTG ACT CAG CCG
VH-5. GAA GTG CAG CTG GTG CAG TCT	VA-6. AAT TTT ATG CTG ACT CAG CCC
VH-6. CAG GTA CAG CTG CAG CAG TCA	VA-7a. CAG ACT GTG GTG ACT CAG CAG
VH-7. CAG GTG CAG CTG GTG CAA TCT	VA-7b. CAG GCT GTG GTG ACT CAG GAG
IgM AAG GGT TGG GGC GGA TGC ACT 38	VA-8. CAG ACT GTG GTG ACC CAG GAG
	VA-9. CAG CCT GTG CTG ACT CAG CCA
	VA-10. CAG GCA GGG CTG ACT CAG CCA
	Vk-1a. GAC ATC CAG ATG ACC CAG TCT
	Vk-1b. AAC ATC CAG ATG ACC CAG TCT
	Vk-1c. GCC ATC CAG TTG ACC CAG TCT
	Vk-1d. GAC ATC CAG TTG ACC CAG TCT
	Vk-1e. GCC ATC CAG ATG ACC CAG TCT
	Vk-1f. GTC ATC TGG ATG ACC CAG TCT
	Vk-1g. GCC ATC CAG ATG ACC CAG TCT
	Vk-2a. GAT ATT GTG ATG ACC CAG ACT
	Vk-2b. GAT GTT GTG ATG ACT CAG TCT
	Vk-2c. GAT ATT GTG ATG ACT CAG TCT
	Vk-3a. GAA ATT GTG TTG ACG CAG TCT
	Vk-3b. GAA ATT GTG ATG ACG CAG TCT
	Vk-3c. GAA ATT GTA ATG ACG CAG TCT
	Vk-4. GAC ATC GTG ATG ACC CAG TCT
	Vk-5. GAA ACG ACA CTC ACG CAG TCT
	Vk-6a. GAA ATT GTG CTG ACT CAG TCT
	Vk-6b. GAT GTT GTG ATG ACA CAG TCT
	C-λ. GGA CCG CCG GAA CAG AGT GAC
	C-κ. GAC AGA TGG TGC AGC CAC AGT 36

Fig. 8

Oligonucleotides used as primers in 2. PCR for introduction of restriction endonuclease recognition sequences (marked in bold style)

	heavy chain primer	light chain primer
	<b>NcoI</b>	<b>MluI</b>
VH-1a.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG CAG TCT <b>7</b>	CCT ACA GAA CCG GTA CAG TCT GTG CTG ACG CAG CCA <b>94</b>
VH-1b.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG CAG TCT	CCT ACA GAA CCG GTA CAG TCT GTG CTG ACG CAG CCG
VH-1c.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTA CAG TCT	CCT ACA GAA CCG GTA CAG TCT GCC CTG ACT CAG CCT
VH-1d.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTA CAG TCT	CCT ACA GAA CCG GTA TCC TAT GAG CTG ACA CAG CCA
VH-1e.	TGG ACG CCC ATG GCG CAG ATC ACC TTG AAG GAG TCT	CCT ACA GAA CCG GTA TCC TCT GAG CTG ACA CAG GAC
VH-2a.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG GAG TCT	CCT ACA GAA CCG GTA TCC TAT GTG CTG ACA CAG CCA
VH-2b.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG GAG TCT	CCT ACA GAA CCG GTA TCC TAT GAG CTG ACA CAG CTA
VH-3a.	TGG ACG CCC ATG GCG GAA GTG CAG CTG GTG GAG TCT	CCT ACA GAA CCG GTA TCC TAT GAG CTG ACA CAG CCA
VH-3b.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG GAG TCT	CCT ACA GAA CCG GTA TCC TAT GTG CTG ACA CAG CCA
VH-3c.	TGG ACG CCC ATG GCG GAG GTG CAG CTG TTG GAG TCT	CCT ACA GAA CCG GTA CTG CCT GTG CTG ACT CAG CCC
VH-4a.	TGG ACG CCC ATG GCG CAG GTG CAG CTG CAG GAG TCG	CCT ACA GAA CCG GTA CAG CTT GTG CTG ACT CAA TCG
VH-4b.	TGG ACG CCC ATG GCG CAG CTG CAG CTG CAG GAG TCG	CCT ACA GAA CCG GTA CAG CCT GTG CTG ACT CAG CCA
VH-4c.	TGG ACG CCC ATG GCG CAG GTG CAG CTG CAG TCG	CCT ACA GAA CCG GTA CAG GCT GTG CTG ACT CAG CCG
VH-5.	TGG ACG CCC ATG GCG GAA GTG CAG CTG GTG CAG TCT	CCT ACA GAA CCG GTA AAT TTT ATG CTG ACT CAG CCC
VH-6.	TGG ACG CCC ATG GCG CAG GTG CAG CTG CAG CAG TCA	CCT ACA GAA CCG GTA CAG ACT GTG GTG ACT CAG GAG
VH-7.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG CAA TCT	CCT ACA GAA CCG GTA CAG GCT GTG GTG ACT CAG GAG
IgM	<b>HindIII</b> TGG GAA AAG CTT AAG GGT TGG GGC GGA TGC ACT <b>93</b>	CCT ACA GAA CCG GTA CAG CCT GTG CTG ACT CAG CCA
		CCT ACA GAA CCG GTA GAC ATC CAG ATG ACC CAG TCT
Vk-1a.		CCT ACA GAA CCG GTA AAC ATC CAG ATG ACC CAG TCT
Vk-1b.		CCT ACA GAA CCG GTA GCC ATC CAG TTG ACC CAG TCT
Vk-1c.		CCT ACA GAA CCG GTA GAC ATC CAG TTG ACC CAG TCT
Vk-1d.		CCT ACA GAA CCG GTA GTC ATC CAG TTG ACC CAG TCT
Vk-1e.		CCT ACA GAA CCG GTA GTC ATC CAG TTG ACC CAG TCT
Vk-1f.		CCT ACA GAA CCG GTA GTC ATC CAG TTG ACC CAG TCT
Vk-1g.		CCT ACA GAA CCG GTA GTC ATC CAG TTG ACC CAG TCT
Vk-2a.		CCT ACA GAA CCG GTA GAT ATT GTG ATG ACC CAG ACT
Vk-2b.		CCT ACA GAA CCG GTA GAT ATT GTG ATG ACC CAG ACT
Vk-2c.		CCT ACA GAA CCG GTA GAT ATT GTG ATG ACC CAG TCT
Vk-3a.		CCT ACA GAA CCG GTA GAA ATT GTG TTG ACG CAG TCT
Vk-3b.		CCT ACA GAA CCG GTA GAA ATT GTG ATG ACG CAG TCT
Vk-3c.		CCT ACA GAA CCG GTA GAA ATT GTG ATG ACG CAG TCT
Vk-4.		CCT ACA GAA CCG GTA GAC ATC GTG ATG ACC CAG TCT
Vk-5.		CCT ACA GAA CCG GTA GAA ACG ACA CTC ACG CAG TCT
Vk-6a.		CCT ACA GAA CCG GTA GAA ATT GTG CTG ACT CAG TCT
Vk-6b.		CCT ACA GAA CCG GTA GAT GTT GTG ATG ACA CAG TCT
C-λ		<b>NotI</b> GGG CCG CAG GGC GGC GGA CCG CCG GAA CAG AGT GAC
C-κ		GGG CCG CAG GGC GGC GGC GAC AGA TGG TGC AGC CAC AGT <b>85</b>

Fig. 9



FACS analysis of clones SA8, SA10 and SA11.  
Binding of indicated scFv's to activated (black curve) and not activated (grey curve) thrombocytes.

Fig. 10

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pEXHAM4/B4

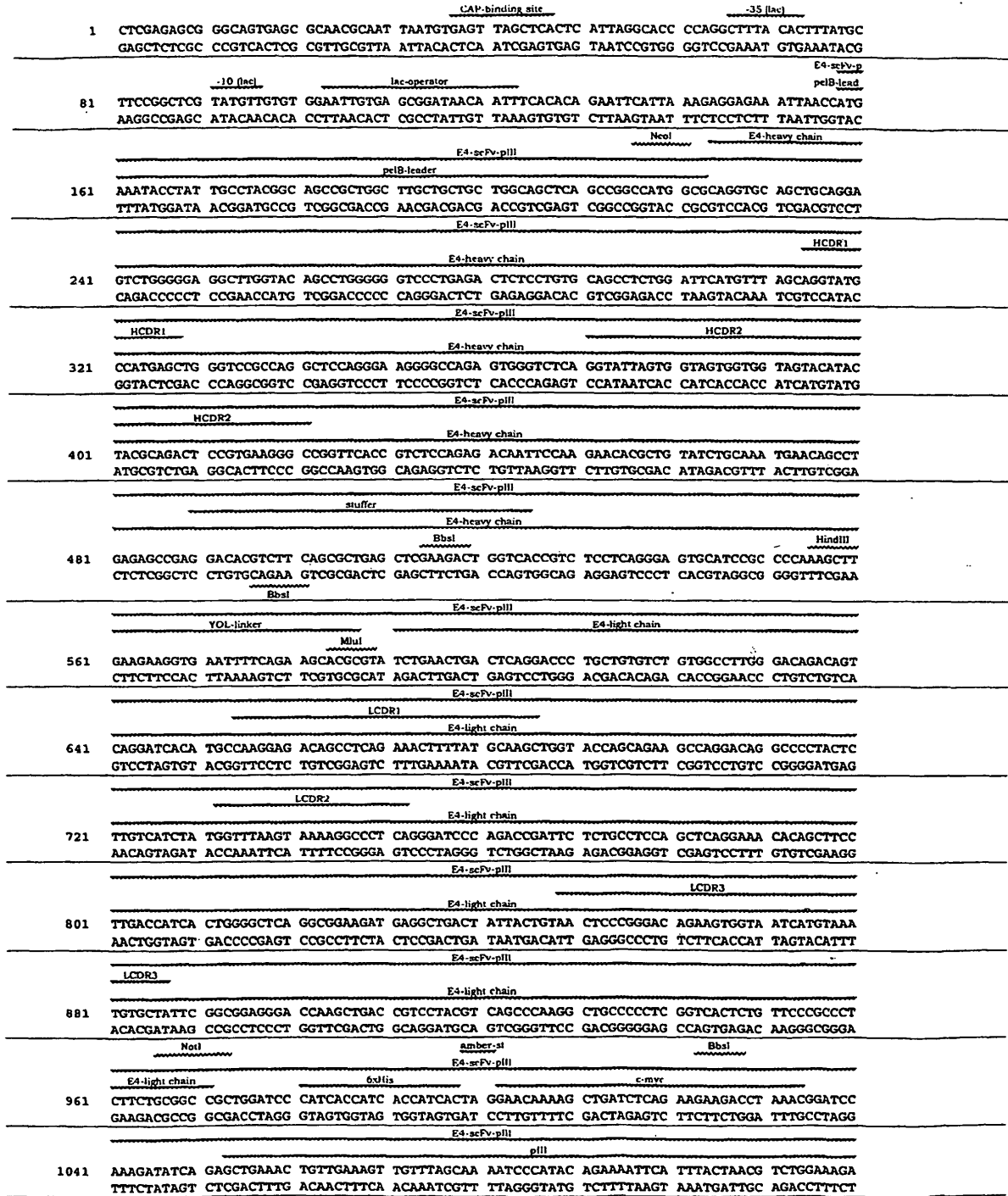


Fig. 11a

pE2HAM4/B4

	E4-scFv-pIII	
	pIII	
1121	CGACAAAAC TTAGATCGTT ACGCTAACTA TGAGGGCTGT CTGTGGAATG CTACAGGCGT TGTAGTTGT ACTGGTGACG	GCTGTTTTGA AATCTAGCAA TGCATTGAT ACTCCGACA GACACCTTAC GATGTCGCA ACATCAAACA TGACCCTGC
	E4-scFv-pIII	
	pIII	
1201	AAACTCAGTG TTACGGTACA TGGGTTCTTA TTGGGCTTGC TATCCCTGAA AATGAGGGTG GTGGCTCTGA GGGTGGCGGT	TTTGAGTCA AATGCCATGT ACCCAAGSAT AACCCGAACG ATAGGGACTT TTACTCCAC CACCAGACT CCCACCSCCA
	E4-scFv-pIII	
	pIII	
1281	TCTGAGGGTG GCGGTTCTGA GGGTGGCGGT ACTAACTC CTGAGTACGG TGATACACCT ATTCGGGGCT ATACTTATAT	AGACTCCCAC CGCCAAGACT CCCACCSCCA TGATTGGAG GACTCATGCC ACTATGTGGA TAAGGCCCGA TATGAATATA
	E4-scFv-pIII	
	pIII	
1361	CAACCTCTC GACGGCACTT ATCCGCTGG TACTGAGCAA AACCCGCTA ATCCTAATCC TTCTTTGAG GAGTCTCAGC	GTGGGAGAG CTGCCGTGAA TAGCGGACC ATGACTCGTT TTGGGGCGAT TAGGATTAGG AAGAGAACT CTCAGACTCG
	E4-scFv-pIII	
	pIII	
1441	CTCTAATAC TTTCATGTTT CAGAATAATA GGTTCGAAA TAGGCAGGGG GCATTAACCTG TTTATACGG CACTGTTACT	GAGAATTATG AAGTACAAA GTCTTATTAT CCAAGGCTTT ATCCGTCGCC CGTAATTGAC AAATATGCC GTGACAATGA
	E4-scFv-pIII	
	pIII	
1521	CAAGGCACTG ACCCGTTAA AACTTATTAC CAGTACACTC CTGTATCATC AAAAGCCATG TATGACGCTT ACTGGAACCG	GTCCGTTGAC TGGGCAATT TTGAATAATG GTCATGTGAG GACATAGTAG TTTTCGGTAC ATACTGCGAA TGACCTTGGC
	E4-scFv-pIII	
	pIII	
1601	TAAATTCAGA GACTGCGCTT TCCATTCTGG CTTAAATGAG GATTTATTG TTTGTGAATA TCAAGGCCAA TCGTCTGACC	ATTTAATCT CTGACGCGAA AGGTAAGACC GAAATFACTC CTAATAAAC AACACTTAT AGTTCCGGTT AGCAGACTGG
	E4-scFv-pIII	
	pIII	
1681	TGCCTCAACC TCCTGTCAAT GCTGGCGGCG GCTCTGGTGG TGGTCTGTGT GCGGCTCTG AGGGTGGTGG CTCTGAGGGT	ACGGAGTGG AGGACAGTTA CGACCSCCGC CGAGACCACC ACCAAGACCA CCGCCGAGAC TCCCACCACC GAGACTCCCA
	E4-scFv-pIII	
	pIII	
1761	GGCGTCTG AGGGTGGCGG CTCTGAGGGA GCGGTTCCG GTGGTGGCTC TGGTCCCGT GATTTGATT ATGAAAAGAT	CCGCAAGAC TCCCACCSCC GAGACTCCCT CCGCCAAGGC CACCACCGAG ACCAAGGCCA CTAATACTAA TACTTTTCTA
	E4-scFv-pIII	
	pIII	
1841	GGCAAACGCT AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCGC TACAGTCTGA CGCTAAAGGC AAACCTGATT	CCGTTTGGGA TTATCCCCC GATCTGGCT TTTACGGCTA CTTTGGCGG ATGTCAGACT GCGATTTCCG TTTGAACTAA
	E4-scFv-pIII	
	pIII	
1921	CTGTGCTAC TGATTACGGT GCTGCTATCG ATGGTTTCA TGGTACGTT TCCGGCCTG CTAATGTTAA TGGTCTACT	GACAGCATG ACTAATGCCA CGACGATAGC TACCAGTA ACCACTGCA AGCCGGGAC GATTACCATT ACCAGATGA
	E4-scFv-pIII	
	pIII	
2001	GGTGATTTG CTGGCTCTAA TTCCAAATG GCTCAAGTGG GTGACGGTGA TAATTCACCT TTAATGAATA ATTTCCGTTA	CCACTAAAC GACCGAGATT AAGGGTTTAC CGAGTTCAGC CACTGCCACT ATTAAGTGA AATTACTTAT TAAAGGCAGT
	E4-scFv-pIII	
	pIII	
2081	ATATTACCT TCCCTCCCTC AATCGGTTGA ATGTGCCCT TTTGTCTTG GCGTGGTAA ACCATATGAA TTTTCTATTG	TATAAATGGA AGGGAGGGAG TTAGCCAAC TACAGCGGA AACAGAAAC CCGACCACT TGTATACTT AAAAATAAC
	E4-scFv-pIII	
	pIII	
2161	ATTGTGCAA AATAAACTA TTCCGTTGG TCTTTGGTT TCTTTATAT GTTGCCACT TTATGTATGT ATTTCTACG	TAACACTGTT TTATTTGAAT AAGGCACCAC AGAAACGCAA AGAAATATA CAACGGTGA AATACATACA TAAAGATGC
	E4-scFv-pIII	
	pIII	
2241	TTTGCTACA TACTGCGTAA TAAGGAGTCT TAATGATCTA GAGGCTGTG CTAATGATCA GCTAGCTTGA GGCATCAATA	AAACGATTGT ATGACGCATT ATTCCTCAGA ATACTAGAT CTCCGGACAC GATTACTAGT CGATGAACT CCGTAGTTAT
2321	AAACGAAAG CTACGTGAA AGACTGGGCC TTTGTTTTA TCTGTTGTTT GTCGGTTAAC GTCGACCTGG CGTAATAGCG	TTTGCTTCC GAGTCAGCTT TCTGACCCGG AAAGCAAAAT AGACAACAAA CAGCCAATTG CAGCTGGACC GCATTATCGC
2401	AAGAGGCCG CACCGATCGC CCTTCCCAAC AGTTGCGCAG CCTGAATGGC GAATGGGACG CGCCCTGTAG CGCGCATT	TTCTCCGGC GTGGCTAGCG GGAAGGGTTG TCAACGCTC GACTTACCG CTTACCCTGC GCGGACATC CCGCGTAAT
2481	AGCGCGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTCCAG CGCCCTAGCG CCGCTCCTT TCGTTTCTT	TCGCGCGCC CACACCACA ATGCGGCTCG CACTGCGGAT GTGAACGCTC GCGGGATCG GCGGAGGAA AGCGAAGAA
2561	CCCTTCTTT CTGCGCACT TCGCGGCTT TCCCGTCAA GCTCTAAATC GGGGGCTCCC TTAGGGTTC CGATTAGTG	GGGAAGGAA GAGCGGTGCA AGCGCGGAA AGGGCAGTT CGAGATTAG CCCCAGGGG AATCCCAAG GCTAAATCAC

Fig. 11b

pEXHAM4/E4

2641	CTTTACGGCA CCTCGACCCC AAAAACTTG ATTAGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT GAAATGCCGT GGAGCTGGGG TTTTTGAAC TAATCCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAA
2721	CGCCCTTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCT AACTGGAAAC AACTCAACC CTATCTCGGT GCGGGAAACT GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAAGT TTGACCTTGT TGTGAGTTGG GATAGAGCCA
2801	CTATTCTTTT GATTTATAAG GGATTTGCC GATTTGGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAAG GATAAGAAA CTAATATTC CCTAAAACGG CTAAGCCGG ATACCAATT TTTTACTCGA CTAATTTGT TTTAATTC
2881	CGAATTTAA CAAATATTA ACGTTACAA TTTAGTGGC ACTTTTCGGG GAAATGTGG CGGAACCCCT ATTGTATT GCTTAAATTT GTTTTATAAT TGCGAATGT AAATCCACCG TGAAGAGCCC CTTTACACGC GCCTGGGGA TAAACAAATA
2961	TTTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA ATAACCTTGA TAAATGCTTC AATAATATTG AAAAAGGAAG AAAAGATTTA TGTAAGTTA TACATAGGCG AGTACTCTGT TATTGGGACT ATTTACGAAG TTATTATAAC TTTTCTCTC
bla	
3041	AGTATGAGTA TTCAACATTT CCGTGTCCGC CTTATTCCCT TTTTGGCGC ATTTGCGCTT CCTGTTTTTG CTCACCCAGA TCATACTCAT AAGTGTAAA GGCACAGCGG GAATAAGGGA AAAAAAGCCC TAAAAAGGAA GGACAAAAAC GAGTGGGTCT
bla	
3121	AACGCTGGTG AAAGTAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA TTGCGACCAC TTTCAATTC TACGACTTCT AGTCAACCCA CGTGCTCACC CAATGTAGCT TGACCTAGAG TTGTCGCCAT
bla	
3201	AGATCCTTGA GAGTTTTGCG CCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC TGCTATGTGG CCGGATTA TCTAGGAACT CTCAAAAGCG GGGCTTCTG CAAAAGGTTA CTACTCTGTA AAATTTCAAG ACGATACACC GCGCCATAAT
bla	
3281	TCCTGTATTG ACGCCGGGCA AGAGCAACT GGTCCGGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT AGGGCATAAC TGCGCCCGT TCTCGTTGAG CCAGCGCGGT ATGTGATAAG AGTCTTACTG AACCAACTCA TGAGTGGTCA
bla	
3361	CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGTAT AACACTGCGG GTGCTTTTC GTAGAATGCC TACCGTACTG TCATTCTCTT AATACGTAC GACGGTATTG GTACTCACTA TTGTGACGCC
bla	
3441	CCAACCTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACCTGC GGTTGAATGA AGACTGTTC TAGCCTCTG GCTTCCCTGA TTGGCGAAAA AACGTGTGT ACCCCCTAGT ACATTGAGCG
bla	
3521	CTTGATGTT GGGAAACCGA GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCAGC ATGCCTGTAG CAATGGCAAC GAAC TAGCAA CCCTTGGCCT CGACTTACTT CGSTATGGT TGCTGCTCGC ACTGTGGTGC TACGGACATC GTTACCGTTC
bla	
3601	AACGTTGCGC AAACATTTAA CTGGCGAACT ACTTACTCTA GCTTCCCGCC AACAAATTAAT AGACTGGATG GAGGCGGATA TTGCAACCGG TTTGATAAAT GACCGCTTGA TGAATGAGAT CGAAGGGCCG TTGTTAATTA TCTGACCTAC CTCGCCCTAT
bla	
3681	AAGTTGACAG ACCACTTCTG CGCTCGGCC TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TTCACCTCC TGGTGAAGAC GCGAGCCGGG AAGGCCGACC GACCAATAAA CGACTATTTA GACCTCGGCC ACTCGCACCC
bla	
3761	TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG GGAGTCAGGC AGAGCCCAT AGTAACCTCG TGACCCCGGT CTACCATTCC GGAGGGCATA GCATCAATAG ATGTGCTGCC CCTCAGTCCG
bla	
3841	AACATGAGAT GAACGAATA GACAGATCGC TGAGATAGGT GCCTCACTGA TTAGCATTG GTAACGTCA GACCAAGTTT TTGATACCTA CTGCTTTAT CTGCTAGCG ACTCTATCCA CGGAGTGACT AATTCGTAAC CATGACAGT CTGGTTCAA
3921	ACTCATATAT ACTTATGATT GATTTAAAAC TFCATTTTAA ATTTAAAGG ATCTAGGTGA AGATCTTTT TGATAATCTC TGAGTATATA TGAATCTAA CTAATTTTG AAGTAAAAT TAAATTTTCC TAGATCCACT TCTAGGAAA ACTATTAGAG
4001	ATGACCAAAA TCCCTTAAAC TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTCTTTGAGA TACTGGTTTT AGGGAATTGC ACTCAAAAGC AAGGTGACTC GCAGTCGGG GCATCTTTT TAGTTTCTTA GAAGAACTCT
4081	TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAAACAAA AAACCACCGC TACCAGCGGT GGTGTGTTG CCGGATCAAG AGGAAAAAAA GACCGCATTT AGACGAAGAA CGTTTGTTTT TTTGGTGGC ATGGTCGCCA CCAACAAAAC GGCCTAGTTC
4161	AGTACCAAC TCTTTTTCG AAGTAACTG GCTTCAGCAG AGCGCAGATA CCAATACTG TCCTTCTAGT GTAGCCGTAG TCGATGGTTG AGAAAAAGGC TTCCATTGAC CGAAGTCTGCT TCGCTCTAT GGTITATGAC AGGAAGATCA CATCGGCATC
4241	TTAGGCCACC ACTTCAAGAA CTCTGTAGCA CCGCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG AATCCGTTGG TGAAGTCTTT GAGACATCGT GCGGATGTA TGGAGCGAGA CGATTAGAC AATGTCACC GACGACGGTC
4321	TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGGCCA CGGTCGGGG TGAACGGGGG ACCGTATTC AGCACAGAAAT GGCCCAACCT GAGTCTGCT ATCAATGGCC TATTCGGGT CGCCAGCCCG ACTTGCCTCC
4401	GTTCGTGCAC ACAGCCACG TTGGAGCGAA CGACCTACAC CGAATGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC CAAGCACGTG TGTCGGGTG AACCTCGCTT GCTGGATGTG GCTTGACTCT ATGGATGTC CACTCGATAC TCTTTCGGG
4481	ACGCTCCCG AAGGGAGAAA GCGGACAGG TATCCGTAA GCGGACGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC TGCGAAGGGC TTCCCTTTT CCGCTGTCC ATAGGCCATT CGCCGTCCA GCCTTGTCTT CTGCGTGTCT CCTCGAAGG
4561	AGGGGGAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACTCT GACTTGAGCG TCGATTTTGT TGATGCTGT TCCCTTTTG CGGACCATAG AAATATCAGG ACAGCCCAA GCGGTGGAGA CTGAACCTCG AGCTAAAAC ACTACGAGCA
4641	CAGGGGGCG GAGCCTATGG AAAACGCCA GCAACGCGC CTTTTACGG TTCCGCGCT TTTGCTGGCC TTTTGTCTAC GTCCTCCCGC CTCGGTATCC TTTTTCGGT CGTTGCGCGG GAAAAATGCC AAGGACCGGA AAACGACCGG AAAACGAGTG
4721	ATGTTCTTTC CTGCGTATC CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGTGATG GCTGATACCG CTCGCGCAG TACAAGAAAG GACGCAATAG GGGACTAAGA CACCTATTGG CATAATGGCG GAAACTCACT CGACTATGGC GAGCGCGCTC

Fig. 11c

pEXHAM4/B4

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4801  CCGAACGACC GAGCCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC CAATACGCAA ACCGCCTCTC CCGCGCGTT  
      GGCTTGCTGG CTCGCGTCGC TCAGTCACTC GCTCCTTCGC CTTCFCGCGG GTTATGCGTT TGGCGGAGAG GGGCGCGCAA  
4881  GGCCGATTCA TTAATGCAGG TATCAGGAG CCCTTTCGTC TTCAC  
      CCGGCTAAGT AATTACGTCC ATAGTGCTCC GGGAAAGCAG AAGTG  
      ~~~~~  
      BbsI
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Fig. 11d

pEXHAM4/C9

1 CTCGAGAGCG GGCAGTGGC GCAACGCAAT TAATGTGAGT TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC  
 GAGCTCTCGC CCGTCACTCG CGTTGCGTTA ATTACATCA ATCGAGTGAG TAATCCGTGG GGTCCGAAAT GTGAAATACG

81 TTCGGCTCG TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTACACA GAATTCATTA AAGAGGAGAA ATTAACCATG  
 AAGGCCGAGC ATACAACACA CCTTAACACT CGCCTATTGT TAAAGTGTGT CTTAAGTAAT TTCTCCTCTT TAATGGTAC

161 AAATACCTAT TGCCTACGGC AGCCGCTGGC TTGCTGTGTC TGGCAGCTCA GCCGGCCATG GCGCAGGTAC AGCTGCAGGA  
 TTTATGGATA ACGGATGCCG TCGGCGACCG AACGACGACG ACCGTGAGT CGGCCGGTAC CGCGTCCATG TCGACGCTCT

241 GTCTGGGGGA GCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCCTCTGG ATTCTCCTTC AGTAATTATG  
 CAGACCCCTT CCGCACCAGG TCGGACCCTC CAGGACTCTG GAGAGGACAC GTCCGGAGACC TAAGAGGAAG TCATTAATAC

321 GCATACACTG GGTCCGCCAG GCTCCAGGCA AGGGGTGGA GTGGGTGGCA CTTATATCAT ATGATGGAAA TAAGAAATTC  
 CGTATGTGAC CCAGGCGGTC CGAGGTCCGT TCCCCGACCT CACCCACCGT GAATATAGTA TACTACCTTT ATTCTTTAAG

401 TATGCAGACT CGTGAAGGG CCGATTGCGC ATCTCCAGAG ACACTTCTAA GAATACGGTG GATCTGCAA TGACCAGCCT  
 ATACGTCTGA GGCACCTCCC GGCTAAGCGG TAGAGGTCTC TGTGAAGATT CTTATGCCAC CTAGACGTTT ACTGGTGGGA

481 GAGACCTGAG GACACGCTCT CAGCGCTGAG CTGGAAGACT GGTACCCGTC TCCTCAGCCT CCACCAAGGG CCCAAAGCTT  
 CTCTGGACTC CTGTGCAGAA GTCGCGACTC GAGCTTCTGA CCAGTGGCAG AGGAGTCGGA GGTGGTTCCC GGGTTTCGAA

561 GAAGAAGGTG AATTTTCAGA AGCACGCGTA TCCTATGAAC TGACTCAGCC ACCCTGGGTG TCAGTGGCCC CAGGACAGAC  
 CTCTCTCCAC TTAAGAGTCT TCGTGCACAT AGGATACTTG ACTGAGTCCG TGGGAGCCAC AGTCACCGGG GTCCGTCTCTG

641 GGCCATGATT ACCTGTGGGG GAAACAACAT TGGAAGTACA ACCGTGCACT GGTATCAGCA GAAGCCAGGC CAGGCCCTTG  
 CCGGACTAA TGGACACCCC CTTTGTGTA ACCTTCAATG TGGCAGGTGA CCATAGTCTG CTTGGTCCG GTCCGGGGAC

721 TGCTGGTCTG CTATGATGAT AACGAGGAC CCTCAGGAT CCTGAGCGA TTCTCTGCTT CCAACTCTGG GAGCACGGCC  
 ACGACCAGCA GATACTACTA TTGCTCGCTG GGAGTCCCTA GGGACTCGCT AAGAGACCGA GGTGAGACC CTCGTCCCGG

801 ACCCTGACCA TCAACAGGGT CGAAGCCGGG GATGAGGCCG ACTATTATG TCAAGTGTGG GATAGTGGTA GTGATCATGT  
 TGGACTGGT AGTTGTCCA GCTTGGGCC CTACTCCGGC TGATAATAAC AGTTCACACC CTATCACCAT CACTAGTACA

881 GGTATTCCGC GGAGGGACGA AGCTGACCGT CTTAGTCTAG CCCAAGGCTG CCCCTCGGT CACTCTGTTT CCGCCCTCT  
 CCATAAGCCG CCTCCCTGCT TCGACTGSCA GGATCCAGTC GGGTCCGAC GGGGGAGCCA GTGAGACAAG GGCGGGAGGA

961 CTGCGGCCGC TGGATCCCAT CACCATCACC ATCACTAGGA ACAAAAGCTG ATCTCAGAAG AAGACCTAAA CCGATCCAAA  
 GACGCGCGGC ACCTAGGGTA GTGGTAGTGG TAGTGATCCT TGTTTTGAC TAGAGTCTTC TTCTGGATTG GCCTAGGTTT

1041 GATATCAGAG CTGAACCTGT TGAAGTGTG TTAGCAAAT CCCATACAGA AAATTCATIT ACTAACGCTT GGAAGACGCA  
 CTATAGTCTC GACTTTGACA ACTTTCAACA AATCGTTTTA GGGTAGTCTT TTAAGTAAA TGATTGCAGA CCTTTCTGCT

Fig. 12a

pEXHAM4/C9

	C9-seFv-pIII	
	pIII	
1121	CAAAACITTA GATCGTTACG CTAACATGA GGGCTGCTG TGGAAATGCTA CAGGCGTGT AGTTTGTACT GGTGACGAAA	GTITTTGAAAT CTAGCAATGC GATTGATACT CCCGACAGAC ACCTTACGAT GTCCGCAACA TCAAACATGA CCAGTGTCTT
	C9-seFv-pIII	
	pIII	
1201	CTCAGTGTTA CGGTACATGG GTTCCTATTG GGCTTGCTAT CCCTGAAAAT GAGGGTGGTG GCTCTGAGGG TGGCGGTTCT	GAGTCACAAT GCCATGTACC CAAGGATAAC COGAAACGATA GGGACTTTTA CTCCACCAC CGAGACTCCC ACCGCCAAGA
	C9-seFv-pIII	
	pIII	
1281	GAGGGTGGCG GTTCTGAGGG TGGCGGTACT AAACCTCCTG AGTACGGTGA TACACCTATT CCGGGCTATA CTTATATCAA	CTCCACCAGC CAAGACTCCC ACCGCCATGA TTTGGAGGAC TCATGCCACT ATGTGGATAA GGCCCGATAT GAATATAGTT
	C9-seFv-pIII	
	pIII	
1361	CCCTCTGAC GGCACITATC CGCCTGGTAC TGAGCAAAC CCGCTAATC CTAATCCTTC TCTTGAGGAG TCTCAGCCTC	GGGAGAGCTG CCGTGAATAG GCGGACCATG ACTCGTTTGG GGGCGATTAG GATTAGGAAG AGAACTCCTC AGAGTCCGAG
	C9-seFv-pIII	
	pIII	
1441	TTAATACTTT CATGTTTCAG AATAATAGGT TCCGAAATAG GCAGGGGGCA TTAAGTGTTT ATACGGGCAC TGTTACTCAA	AATTATGAAA GTACAAAGTC TTATTATCCA AGGCTTTATC CGTCCCGCT AATTGACAAA TATGCCCGTG ACAATGAGTT
	C9-seFv-pIII	
	pIII	
1521	GGCACTGACC CCGTAAAAAC TTATTACCAG TACACTCCTG TATCATCAA AGCCATGTAT GACGCTTACT GGAACGGTAA	CCGTGACTGG GCAATTTTGG AATAATGGTC ATGTGAGGAC ATAGTAGTIT TCGGTACATA CTGCGAATGA CCTTGCCATT
	C9-seFv-pIII	
	pIII	
1601	ATTGAGAGAC TGCGCTTCC ATTCTGGCTT TAATGAGGAT TTATTGTITT GTGAATATCA AGCCCAATCG TCTGACCTGC	TAAGTCTCTG ACCGCAARAG TAAGACCGAA ATTACTCTTA AATAAACAAA CACTTATAGT TCCGGTTAGC AGACTGGACG
	C9-seFv-pIII	
	pIII	
1681	CTCAACTCC TGCAATGCT GCGCGCGSCT CTGGTGGTGG TTCTGGTGGC GGCTCTGAGG GTGGTGGCTC TGAGGGTGGC	GAGTTGGAGG ACAGTTACGA CCGCCGCGA GACCACCACC AAGACCACC CCGAGACTCC CACCACCGAG ACTCCACCAG
	C9-seFv-pIII	
	pIII	
1761	GGTTCTGAGG GTGGCGGCTC TGAGGGAGGC GGTTCGGGTG GTGGCTCTGG TTCCGGTGAT TTTGATTATG AAAAGATGGC	CCAAGACTCC CACCGCCGAG ACTCCCTCCG CCAAGGCCAC CACCGAGACC AAGGCCACTA AAATAATAC TTTTCTACCG
	C9-seFv-pIII	
	pIII	
1841	AAACGCTAAT AAGGGGGCTA TGACCGAAAA TGCCGATGAA AACCGCTAC AGTCTGACGC TAAAGGCAAA CTGTATTCTG	TTTGGGATTA TTCCCGCAT ACTGGCTTTT ACGGCTACTT TTGGCGATG TCAGACTGCG ATTTCCGTTT GAACTAAGAC
	C9-seFv-pIII	
	pIII	
1921	TGCTACTGTA TTACGGTGTCT GCTATCGATG GTTTCATTGG TGAAGTITCC GGCCITGCTA ATGGTAATGG TGCTACTGGT	AGCGATGACT AATGCCACGA CGATAGCTAC CAAAGTAACC ACTGCAAAG CCGGAACGAT TACCATTACC ACGATGACCA
	C9-seFv-pIII	
	pIII	
2001	GATTTTGTCT GCTCTAATTC CCAAATGGCT CAAGTCGGTG ACGGTGATAA TTCACCTTA ATGAATAATT TCCGTCAATA	CTAAAACGAC CGAGATTAAG GGTTCACCGA GTTCAGCCAC TGCCACTATT AAGTGGAAAT TACTTATTA AGGCAGTTAT
	C9-seFv-pIII	
	pIII	
2081	TTTACCTTCC CTCCCTCAAT CGGTGGAATG TCGCCCTTT GTCTTTGGCG CTGGTAAACC ATATGAATTT TCTATGTATT	AAATGGAAGG GAGGGAGTTA GCCAACTTAC AGCGGGAATA CAGAAACCGC GACCATTGG TATACTTAA AGATAACTAA
	C9-seFv-pIII	
	pIII	
2161	GTGACAAAAT AAACITATTC CGTGGTGTCT TTGGCTTCT TTATATGTT GCCACCTTA TGTATGTATT TTCTACGTTT	CACGTGTTTA TTTGAATAAG GCACCACAGA AACGCAAAGA AAATATACAA CCGTGGAAAT ACATACATAA AAGATGCAAA
	C9-seFv-pIII	
	pIII	
2241	GCTAACATAC TGCGTAATAA GGAGTCTTAA TGATCTAGAG GCCTGTGCTA ATGATCAGCT AGCTTGAGGC ATCAATAAAA	CGATTGTATG ACGCATTATT CCTCAGAATT ACTAGATCTC CGGACAGGAT TACTAGTCTGA TCGAACTCCG TAGTTATTTT
2321	CGAAAGGCTC AGTCGAAAGA CTGGGCCTTT CGTTTTATCT GTTGTITGTC GGTAAACGTC GACCTGGCGT AATAGCGAAG	GCTTTCCGAG TCAGCTTCTT GACCCGAAA GCAAAATAGA CAACAAACAG CCAATTGCAG CTGGACCGCA TTATCGCTTC
2401	AGGCCCGCAC CGATCCCGCT TCCCAACAGT TGCGCAGCCT GAATGGCGAA TGGGACGCGC CCGTAGCGG CGCATTAAGC	TCCGGCGGTG GCTAGCGGGA AGGGTTGTCA ACGGCTCGGA CTTACCGCTT ACCCTGCGCG GGACATCGCC GCGTAATTCG
2481	GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC TTGCCAGCGC CTTAGCGCCC GCTCCTTTCC CTTCTTCCC	CGCCGCCAC ACCACCAATG CGCGTCCGAC TGGCGATGTC AACGGTCCGCG GGATCGCGGG CGAGGAAAGC GAAAGAAGGG
2561	TTCTTTCTC GCCACGTTCC CCGGCTTCC CCGTCAAGCT CTAATTCGGG GGCTCCCTTT AGGGTTCCGA TTTAGTGTCT	AAGGAAAGAG CCGTGCARAG GCGGAAAGG GGCAGTTCSA GATTTAGCCC CCGAGGGAAA TCCCAAGGCT AAATCACGAA

Fig. 12b

pEXHAM4/C9

2641	TACGGCACCT	CGACCCCAA	AAACTGATT	AGGGTATGG	TTCACGTAGT	GGGCCATCGC	CCTGATAGAC	GGTTTTTCGC
	ATGCCGTGGA	GCTGGGGTTT	TTTGAATAA	TCCCCTACC	AAGTGCATCA	CCCGGTAGCG	GGACTATCTG	CCAAAAGCG
2721	CCTTTGACGT	TGGAGTCCAC	GTTCTTTAAT	AGTGGACTCT	TGTTCCAAAC	TGGAACAACA	CTCAACCCCTA	TCTCGGTCTA
	GGAAACTGCA	ACCTCAGGTG	CAAGAAATTA	TCACCTGAGA	ACAAGGTTTG	ACCTTGTGTG	GAGTTGGGAT	AGAGCCAGAT
2801	TTCTTTTGAT	TTATAAGGGA	TTTTGCCGAT	TTCGGCCTAT	TGGTTAAAAA	ATGAGCTGAT	TTAACAAAAA	TTAACCGCA
	AAGAAAACTA	AATATTCCTT	AAAAACGCTA	AAGCCGGATA	ACCAATTTTT	TACTCGACTA	AATGTTTTTT	AAATFGCGCT
2881	ATTTTAAACA	AATATTAACG	CTTACAATTT	AGGTGGCACT	TTTTGGGGAA	ATGTGCGCGG	AACCCCTATT	TGTTTTTTTT
	TAAAATTGTT	TTATAATTGC	GAATGTTAAA	TCCACCGTGA	AAAGCCCTT	TACACGCGCC	TTGGGGATAA	ACAAATAAAA
2961	TCTAAATACA	TTCAAATATG	TATCGCTCA	TGAGACAATA	ACCCTGATAA	ATGCTTCAAT	AATATTGAAA	AAGGAGAGT
	AGATTTATGT	AAGTTTATAC	ATAGCGGAGT	ACTCTGTIAT	TGGGACTATT	TACGAAGTGA	TTATAACTTT	TTCTTCTCA
					bla			
3041	ATGAGTATTC	AACATTTCCG	TGTGCCCCTT	ATTCCCTTTT	TTGGGGCATT	TTGCCCTCCT	GTTTTTTGCTC	ACCCAGAAAC
	TACTCATAAG	TTGTAAGGCG	ACAGCGGGAA	TAAGGGAAAA	AACCCCGTAA	AACGGAAAGG	CAAAAACGAG	TGGGTCTTTG
					bla			
3121	GCTGGTGAAA	GTAAGGATG	CTGAAGATCA	GTGGGTGCA	CGAGTGGGTT	ACATCGAACT	GGATCTCAAC	AGCGGTAAAG
	CGACCACCTT	CATTTCTAC	GACTTCTAGT	CAACCACCGT	GCTCACCCAA	TGTAGCTTGA	CCTAGAGTTG	TCCGCATTCT
					bla			
3201	TCCTTGAGAG	TTTTCGCCCC	GAAGAACGTT	TTCCAATGAT	GAGCACTTTT	AAAGTCTGCG	TATGTGGCGC	GGTATTATCC
	AGGAACTCTC	AAAAGCGGGG	CTTCTGCAA	AAGGTTACTA	CTCGTGAATA	TTCAAGACG	ATACACCGCG	CCATAATAGG
					bla			
3281	CGTATTGAGC	CCGGGCAAGA	GCAACTCGGT	CGCCGCATAC	ACTATTCTCA	GAATGACTTG	GTTGAGTACT	CACCAGTCAC
	GCATAACTGC	GGCCCGTTCT	CGTTGAGCCA	GCGCGGTATG	TGATAAGAGT	CTTACTGAAC	CAACTCATGA	TGTGTCAGTG
					bla			
3361	AGAAAAGCAT	CTTACGGATG	GCATGACAGT	AAGAGAATTA	TGCAGTGCTG	CCATAACCAT	GAGTGATAAC	ACTGCGGCCA
	TCTTTTCGTA	GAATGCCTAC	CGTACTGTCA	TTCTCTTAAT	ACGTCAACGAC	GGTATTGATA	CTCACTATTG	TGACGCCGGT
					bla			
3441	ACTTACTTCT	GACACAGATC	GGAGGACCGA	AGGAGCTAAC	CGCTTTTTTG	CACAACATGG	GGGATCATGT	AACTCGCCTT
	TGAATGAAGA	CTGTGTCTAG	CCTCCTGGCT	TCCTCGATTG	CGGAAAAAAC	GTGTGTGACC	CCCTAGTACA	TTGAGCGGAA
					bla			
3521	GATCGTTGGG	AACCGGAGCT	GAATGAAGCC	ATACCAAACG	ACGAGCGTGA	CACCACGATG	CCTGTAGCAA	TGGCAACAAC
	CTAGCAACCC	TGGCCCTCGA	CTTACTTCGG	TATGGTTTGG	TGCTCGCACT	GTGGTGCTAC	GGACATCGTT	ACCGTGTGTT
					bla			
3601	GTTCGCGAAA	CTATTAAGTG	GCGAAGTACT	TACTCTAGCT	TCCCGGCAAC	AATTAATAGA	CTGGATGGAG	GCGGATAAAG
	CAACCGGTTT	GATAATTGAC	CGCTTGATGA	ATGAGATCGA	AGGGCCGTTG	TTAATTATCT	GACCTACCTC	CGCCTATTTT
					bla			
3681	TTGCAGGACC	ACITCTGGCG	TCCGCCCTTC	CGSCTGGCTG	GTTTATTGCT	GATAAATCTG	GAGCCGGTGA	CGGTGGGTCT
	AACGTCTCTG	TGAAGACGCG	AGCCGGGAAG	GCCGACCGAC	CAATAACGGA	CTATTTAGAC	CTCGCCACT	CGCACCCAGA
					bla			
3761	CGCGGTATCA	TTGCAGCACT	GGGGCCAGAT	GGAAGCCCT	CCCGTATCGT	AGTTATCTAC	ACGACGGGGA	GTCAGGCAAC
	GCGCCATAGT	AACGTCTGTA	CCCCTGCTTA	CCATTCCGGG	GGGCATAGCA	TCAATAGATG	TGCTGCCCTT	CAGTCCGTTG
					bla			
3841	TATGGATGAA	CGAAATAGAC	AGATCGCTGA	GATAGGTGCC	TCACTGATTA	AGCATTGGTA	ACTGTCAGAC	CAAGTTTACT
	ATACCTACTT	GCTTTATCTG	TCTAGCGACT	CTATCCACGG	AGTGAATAAT	TGTAACCAT	TGACAGTCTG	GTTCAAATGA
3921	CATATATACT	TTAGATTGAT	TTAAAACCTC	ATTTTTAAT	TAAAAGGATC	TAGGTGAAGA	TCCTTTTTGA	TARTCTCATG
	GTATATATGA	AATCTAACTA	AATTTTGAAG	TAAAAATTA	ATTTTCTAG	ATCCACTTCT	AGGAAAAACT	ATTAGAGTAC
4001	ACCAAAATCC	CTTAACGTGA	GTPTTCTGTC	CACGTAGCGT	CAGACCCCGT	AGAAAAGATC	AAAGGATCTT	CCTGAGATCC
	TGGTTTTAGG	GAATTCACCT	CAAAAGCAAG	GTGACTCGCA	GTCTGGGGCA	TCTTTTCTAG	TTCTCTAGAA	GAACCTTAGG
4081	TTTTTTTCTG	CGCGTAATCT	GCTGCTTGA	AACAAAAAAA	CCACCGCTAC	CAGCGGTGGT	TTGTTTGCCG	GATCAAGAGC
	AAAAAAGAC	GCGCATTAGA	CGACGAACGT	TTGTTTTTTT	GGTGGCGATG	GTCCGCCCA	AACAAAACGG	CTAGTTCTCG
4161	TACCAACTCT	TTTTCCGAAG	GTAACGTGCT	TCAGCAGAGC	GCAGATACCA	AATACTGTCC	TTCTAGTGA	GCCGTAGTTA
	ATGGTTGAGA	AAAAGGCTTC	CATGACCGGA	AGTCGTCTCG	CGTCTATGGT	TTATGACAGG	AAGATCACAT	CGGCATCAAT
4241	GGCCACCCT	TCAAGAACTC	TGTAGCACCG	CCTACATACC	TCGCTCTGCT	AATCTGTGA	CCAGTGGCTG	CTGCCAGTGG
	CCGGTGGTGA	AGTTCTTGAG	ACATCGTGGC	GGATGTATGG	AGCGAGACGA	TTAGGACAAT	GGTCACCGAC	GACGGTCAAC
4321	CGATAAGTGG	TGTTTACCG	GGTTGGACTC	AAGACGATAG	TTACCGGATA	AGGGCAGCG	GTCCGGCTGA	ACGGGGGTTT
	GCTATTGAGC	ACAGAATGGC	CCAACCTGAG	TTCTGCTATC	AATGGCTAT	TCCGCTCGC	CAGCCCGACT	TGCCCCCAA
4401	CGTGACACA	GCCCAGCTTG	GAGCGAACGA	CCTACACCGA	ACTGAGATAC	CTACAGCGTG	AGCTATGAGA	AAGCGCCACG
	GCACGTGTGT	CGGGTGAAC	CTCGTGTGCT	GGATGTGGCT	TGACTCTATG	GATGTGCGAC	TCGATACTCT	TTGCGGTGTC
4481	CTTCCGAAG	GGAGAAAGCC	GGACAGGTAT	CCGGTAAAGC	GCAGGGTCGG	AACAGGAGAG	CGCACGAGGG	AGCTTCCAGG
	GAAGGGCTTC	CCTCTTCCG	CCTGTCCATA	GGCCATTCGC	CGTCCCAGCC	TTGTCTCTC	CGGTGCTGCC	TGGAAGGTCC
4561	GGGAAACGCC	TGGTATCTTT	ATAGTCTGTT	CGGGTTTCGC	CACCTCTGAC	TTGAGCGTGG	ATTTTTGTA	TGCTCGTCAG
	CCCTTTGCGG	ACCATAGAAA	TATCAGGACA	GCCCAAAGCG	GTGGAGACTG	AACTCGCAGC	TAAAAACT	ACGAGCAGTC
4641	GGGGCGGAG	CCTATGAAA	AACGCCAGCA	ACGCGGCCTT	TTTACGGTTC	CTGGCCTTTT	GCTGGCCTTT	TGCTCACATG
	CCCCCGCTC	GGATACCTTT	TTGCGTCTGT	TGCGCCGAA	AAATGCCAAG	GACCGGAAA	CGACCGGAAA	ACGAGTGTAC
4721	TTCTTCTCTG	CGTATCCCG	TGATTTCTGT	GATAACCGTA	TTACCGCCTT	TGAGTGAAGT	GATACCGCTC	GCCGACCGG
	AAGAAAGGAC	GCAATAGGGG	ACTAAGACAC	CTATTGGCAT	AATGGCGGAA	ACTCACTCGA	CTATGGCGAG	CGGCGTCCG

Fig. 12c

pEXHAM4/C9

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4801 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCAA TAGCCAAACC GCCTCTCCC GCGCGTTGGC  
TTGCTGGCTC GCGTCGCTCA GTCACTCGCT CCTTCGCCTT CTCGCGGGT ATCGGTTGG CGGAGAGGGG CGCGCAACCG  
4881 CGATTCATTA ATGCAGGTAT CACGAGGCC TTTCGTCTTC AC  
GCTAAGTAAT TACGTCCATA GTGCTCGGG AAAGCAGAAG TG  
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EcoI
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Fig. 12d

pEXHAM7/B4

1 CTGAGAGCG GGCAGTGAGC GCAACGCAAT TAATGTGAGT TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC  
 GAGCTCTCGC CCGTCACTCG CGTTCGTTA ATTACACTCA ATCGAGTGAG TAATCCGTGG GTGCCGAAT GTGAAATACG

81 TTCCGGCTCG TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTACACA GAATTCATTA AAGAGGAGAA ATTAACCATG  
 AAGGCCGAGC ATACAACACA CCTTAACACT CGCTATTGT TAAAGTGTGT CTTAAGTAAT TTCTCCTCTT TAATTGGTAC

161 AAATACCTAT TGCTACGGC AGCCGCTGGC TTGCTGCTGC TGGCAGCTCA GCCGGCCATG GCGCAGGTGC AGCTGCAGGA  
 TTTATGGATA ACGGATGCCG TCGGCGACCG AACGACGAGC ACCGTCGAGT CGGCCGGTAC CGCGTCCAGC TCGACGTCCT

241 GTCTGGGGGA GGCTTGGTAC AGCCTGGGGG GTCCTGAGA CTCTCTGTG CAGCCTCTGG ATTTCATGTTT AGCAGGTATG  
 CAGACCCCCT CCGAACCATG TCGGACCCCC GAGGACTCTT GAGAGGACAC GTCGGAGACC TAAGTACAAA TCGTCCATAC

321 CCATGAGCTG GGTCCGCCAG GCTCCAGGGA AGGGGCCAGA GTGGGTCTCA GGTATTAGTG GTAGTGGTGG TAGTACATAC  
 GGTACTCGAC CCAGGCGGTC CGAGGTCCCT TCCCGGTCT CACCCAGAGT CCATAATCAC CATCACCACC ATCATGTATG

401 TACGCAGACT CCGTGAAGGG CCGGTTCACT GTCTCCAGAG ACAATTCCAA GAACACGCTG TATCTGCAA TGAACAGCCT  
 ATGCGTCTGA GGCACCTCCC GGCCAAGTGG CAGAGTCTC TGTTAAGGTT CTGTGCGAC ATAGACGTTT ACTTGTCCGA

481 GAGAGCCGAG GACACGTCTT CAGCGCTGAG CTGGAAGACT GGTCCCGTTC TCCTCAGGGA GTGCATCCGC CCCAAAGCTT  
 CTCTCGGCTC CTGTGCAGAA GTCGCGACTC GAGCTTCTGA CCAGTGGCAG AGGAGTCCCT CACGTAGGCG GGGTTTCGAA

561 GAAGAAGGTG AATTTTACA AGCAGCGGTA TCTGAACTGA CTCAGGACCC TGCTGTGTCT GTGGCCTGG GACAGACAGT  
 CTCTTCCAC TTAAGAATCT TCGTGCSCAT AGACTTGACT GAGTCTGGG ACGACACAGA CACCGGAACC CTGTCTGTCA

641 CAGGATCACA TGCCAAGGAG ACAGCCTCAG AAACITTTAT GCAAGCTGGT ACCAGCAGAA GCCAGGACAG GCCCTACTC  
 GTCCTAGTGT ACGGTTCTCT GTGCGGAGTC TTTGAAAATA CSTTCGACCA TGTGCTCTT CGSTCCTGTC CCGGGATGAG

721 TTGTCACTA TGGTTAAGT AAAAGGCCCT CAGGGATCCC AGACCGATTC TCTGCCTCCA GCTCAGGAAA CACAGCTTCC  
 AACAGTAGAT ACCAAATCA TTTCCGGGA GTCCCTAGGG TCTGGCTAAG AGACGGAGGT CGAGTCTTT GTGTCGAAGG

801 TTGACCATCA CTGGGGCTCA GCGGAAGAT GAGGCTGACT ATTACTGTAA CTCCGGGAC AGAAGTGGTA ATCATGTAAA  
 AACTGGTAGT GACCCGAGT CCGCCTTCTA CTCCGACTGA TAATGACATT GAGGSCCTG TCTTACCAT TAGTACATTT

881 TGTGCTATTC GCGGAGGGA CCAAGCTGAC CGTCTACGT CAGCCCCAAG CTGCCCCCTC GGTCACTCTG TTCCCGCCT  
 ACACGATAAG CCGCTCCCT. GTTTCGACTG GCAGGATGCA GTCGGTTC GACGGGGGAG CCACTGAGAC AAGGGCGGGA

961 CTTCTGCGGC CGCTGGATCC CATCACCATC ACCATCACTA GGAACAAAAG CTGATCTCAG AAGAGGACCT AAACGGATCC  
 GAAGACGCGG GCGACCTAGG GTAGTGGTAG TGGTAGTAT CTTTGTTC GACTAGAGTC TTCTCTGGA TTGCTTAGG

1041 AAAGATATCA GAGCTGAAAC TGTGAAAGT TGTTTAGCAA AATCCATAC AGAAAATCA TTTACTAACG TCTGGAARAGA  
 TTTCTATAGT CTCGACTTTC ACAACTTCA ACAAACTGT TTAGGTATG TCTTTAAGT AATGATTGC AGACCTTCT

1121 CGACAAAAC TTAGATCGTT ACGCTAATA TGAGGGCTGT CTGTGGAATG CTACAGGCGT TGTAGTTTGT ACTGGTGACG  
 GCTGTTTGA AATCTAGCAA TCGATTGAT ACTCCCGACA GACACCTTAC GATGTCGCA ACATCAAACA TGACCACCTGC

1201 AAACCTAGTG TTACGGTACA TGGGTTCTTA TTGGCTTGC TATCCCTGAA AATGAGGGTG GTGGCTCTGA GGGTGGCGGT  
 TTTGAGTAC AATGCCATGT ACCCAAGGAT AACCCGAACG ATAGGGACTT TTACTCCAC CACCGAGACT CCCACGCCA

1281 TCTGAGGGTG GCGGTTCTGA GGGTGGCGGT ACTAAACCTC CTGAGTACGG TGATACACCT ATTCCGGGCT ATACTTATAT  
 AGACTCCAC CGCCAAGACT CCCACGCCA TGATTTGGAG GACTCATGCC ACTATGTGGA TAAGGCCCGA TATGAATATA

1361 CAACCTCTC GACGGCACTT ATCCGCTGG TACTGAGCAA AACCCGCTA ATCCTAATCC TTCTCTGAG GAGTCTCAGC  
 GTTGGGAGAG CTGCCGTGAA TAGGCGGACC ATGACTCGTT TTGGGGGAT TAGGATTAGG AAGAGAATC CTCAGAGTGC

1441 CTCTTAATC TTTTCATGTT CAGAATAATA GGTTCGAAA TAGGCAGGGG GCATTAACCTG TTTATACGG CACTGTTACT  
 GAGAATTATG AAAGTACAAA GTCTTATAT CCAAGGCTT ATCCGTCACC CGTAATGAC AAATATGCC GTGACAATGA

Fig. 13a

pEXHAM7/B4

	pIII							
1521	CAAGGCACTG	ACCCCGTTAA	AACCTATTAC	CAGTACACTC	CTGTATCATC	AAAAGCCATG	TATGAGCCTT	ACTGGAACGG
	GTTCGGTAC	TGGGCAATT	TTGAATAATG	GTCAITGTAG	GACATAGTAG	TTTTCGGTAC	ATACTGCGAA	TGACCTTGCC
	pIII							
1601	TAAATTCAGA	GACTGCGCTT	TCCATTCTGG	CITTAATGAG	GAITTTATTTG	TTTGTAATA	TCAAGGCCAA	TCGCTGACC
	ATTTAAGTCT	CTGACCGCAA	AGGTAAGACC	GAATTAICTC	CTAAATAAAC	AAACACTTAT	AGTTCCGGTT	AGCAGACTGG
	pIII							
1681	TGCCFCAACC	TCCTGTCAAT	GCTGCGCGG	GCTCTGTGG	TGGTCTCGT	GGCGGCTCTG	AGGGTGGTGG	CTCTGAGGGT
	ACGGAGTTGG	AGGCAGGTA	CGACCGCCG	CGAGACCACC	ACCAAGACCA	CGCCCGAGAC	TCCCACCACC	GAGACTCCCA
	pIII							
1761	GGCGGTTCTG	AGGGTGGCGG	CTCTGAGGGA	GGCGGTTCCG	GTGGTGGCTC	TGGTCCCGT	GATTTTGATT	ATGAAAAGAT
	CCGCCAAGAC	TCCCACCGCC	GAGACTCCCT	CGCCCAAGGC	CACCACCGAG	ACCAAGGCCA	CTAAAACATA	TACTTTCTCA
	pIII							
1841	GGCAACGCT	AATAAGGGG	CTATGACCGA	AAATGCGGAT	GAAAACGCGC	TACAGTCTGA	CGCTAAAGGC	AAACTTGATT
	CCGTTTGGCA	TTATTCCTCC	GATACTGGCT	TTTACGGCTA	CTTTTGGCGG	ATGTCAGACT	GGGATTTCCG	TTTGAACATA
	pIII							
1921	CTGTGCTAC	TGATTAAGGT	GCTGCTATCG	ATGGTTTCAT	TGGTGACGTT	TCCGGCCTTG	CTAATGGTAA	TGGTGTACT
	GACAGCGATG	ACTAATGCCA	CGACGATAGC	TACCAAAGTA	ACCACCTGCA	AGGCCGGAAC	GATTACCATT	ACCACGATGA
	pIII							
2001	GGTGATTTTG	CTGGCTCTAA	TTCCAAATG	GCTCAAGTCG	GTGACGGTGA	TAATTCACCT	TTAATGAATA	ATTTCCGTCA
	CCACTAAAAC	GACCGAGATT	AAGGGTTTAC	CGAGTTCAGC	CAGTCCCACT	ATTAAGTGA	AATTACTTAT	TAAAGGCAGT
	pIII							
2081	ATATTTACCT	TCCCTCCCTC	AATCGGTTGA	ATGTGCGCCT	TTTGTCTTTG	CGCGTGGTAA	ACCATATGAA	TTTTCTATTG
	TATRAATGGA	AGGGAGGGAG	TTAGCCAATC	TACAGCGGGA	AAAACAGAAC	CGCGACCACT	TGGTATACTT	AAAAGATAAC
	pIII							
2161	ATGTGACAA	AATAAACTTA	TTCGGTGGTG	TCCTTGGGTT	TCCTTTATAT	GTGCCACCT	TTATGTATGT	ATTTTCTACG
	TAACACTGTT	TTATTTGAAT	AGGCACCAC	AGAAACGCAA	AGAAAATATA	CAACGGTGA	AATACATACA	TAAAGATGC
	pIII							
2241	TTTGCTAACA	TACTGCGTAA	TAAGGAGTCT	TAATGATCTA	GAGGCCTGTG	CTAATGATCA	GCTAGCTTGA	GGCATCAATA
	AAACGATTTG	ATGACGCATT	ATTCTCTAGA	ATTACTAGAT	CTCCGGACAC	GATTACTAGT	CGATCGAACT	CCGTAGTTAT
2321	AAACGAAAGG	CTCAGTCGAA	AGACTGGGCC	TTTCGTTTTA	TCTGTGTGTT	GTGGTTAAC	GTGACCTGG	CGTAATAGCG
	TTTGCTTTCC	GAGTCAGCTT	TCTGACCCGG	AAAGCAAAAT	AGACAAACA	CAGCCAATG	CAGCTGGACC	GCATTATCGC
2401	AAGAGGCCCG	CACCGATCGC	CCTTCCCAAC	AGTTGCGCAG	CCTGAATGGC	GAATGGGACG	CGCCCTGTAG	CGCGCATTA
	TTCTCCGGGC	GTGGCTAGCG	GGAAGGTTG	TCAACGCGTC	GGACTTACCG	CTTACCCTGC	CGGGACATC	GGCGGTAAT
2481	AGCGCGCGG	GTGTGGTGGT	TACGCGCAGC	GTGACGCTA	CAGTTGCCAG	CGCCCTAGCG	CCCCTCCTT	TCGCTTCTT
	TCGCGCCGCC	CACACCACA	ATGCGCGTGC	CAGTGGCGAT	GTGAACGGTC	CGGGATCGC	GGCGAGGAA	AGCGAAGAA
2561	CCCTTCCCTT	CTCGCCAGT	TGCGCGGCTT	TCCCGTCAA	GCTCTAAATC	GGGGCTCC	TTTAGGTTT	CGATTTAGTG
	GGGAGGAAA	GAGCGGTGCA	AGCGCGGAA	AGGGGCAIT	CGAGATTTAG	CCCCGAGG	AAATCCCAAG	GCTAAATCAC
2641	CTTTAGGCA	CCTCGACCC	AAAAAACTTG	ATTAGGGTGA	TGGTTACGTT	AGTGGCCAT	CGCCCTGATA	GAGGGTTTT
	GAAATGCCGT	GGAGCTGGGG	TTTTTTGAAC	TAATCCCACT	ACCAAGTGA	TCACCCGGTA	GCGGACTAT	CTGCCAATA
2721	CGCCCTTTGA	CGTTGGAGTC	CAGCTTCTT	AATAGTGGAC	TCTTGTCCA	AACTGGAACA	ACACTCAACC	CTATCTCGGT
	CGGGAAACT	GCAACCTCAG	GTGCAAGAAA	TTATCACCTG	AGAACAAGGT	TTGACCTTGT	TGTGAGTTGG	GATAGAGCCA
2801	CTATCTTTT	GATTTATAAG	GGATTTTGGC	GATTTGGGCC	TATTTGTTAA	AAAATGAGCT	GATTTAACAA	AAATTTAAGC
	GATAAGAAA	CTAAATATTC	CCTAAAACGG	CTAAGCCGG	ATAACCAATT	TTTTACTCGA	CTAAATTTGT	TTTAAATTCG
2881	CGAATTTTAA	CAAAATATTA	AGCTTACAA	TTTAGGTGGC	ACTTTTCGGG	GAAATGTGGC	CGGAACCCCT	ATTTGTTTAT
	GCTTAAATTT	GTTTTATAAT	TGCGAATGTT	AAATCCACCG	TGAAAAGCCC	CTTACACGCG	GCCTGGGGA	TAAACAAATA
2961	TTTTCTAAAT	ACATTCAAAT	ATGTATCCGC	TCATGAGACA	ATAACCCCTGA	TAAATGCTTC	AATAATATTG	AAAAGGAAAG
	AAAAGATTTA	TGTAAGTTTA	TACATAGGCG	AGTACTCTGT	TATTTGGACT	ATTTACGAAG	TTATTATAAC	TTTTCTCTC
	bla							
3041	AGTATGAGTA	TTCAACATTT	CGTGTGCGC	CTTATTCCCT	TTTTTGGCGC	ATTTGCTT	CCTGTTTTTG	CTCACCCAGA
	TCATACTCAT	AAGTTGTAAA	GGCACAGCGG	GAATAAGGGA	AAAAACGCCG	TAAAACGGAA	GGACAAAAC	GAGTGGGTCT
	bla							
3121	AACGCTGGTG	AAAGTAAAG	ATGCTGAAGA	TCAGTTGGGT	GCACGAGTGG	GTTACATCGA	ACTGGATCTC	AAAGCGGTA
	TTGCGACCAC	TTTCAATTTT	TACGACTTCT	AGTCAACCCA	CGTGCTCACC	CAATGTAGCT	TGACCTAGAG	TTGTCCCAT
	bla							
3201	AGATCTTGA	GAGTTTTGCG	CCCGAAGAAC	GTTTTCCAAT	GATGAGCACT	TTTAAAGTTC	TGCTATGTGG	CGCGGTATTA
	TCTAGGAACT	CTCAAAAGCG	GGGCTTCTTG	CAAAAGGTTA	CTACTCGTGA	AAATTTCAAG	ACGATACACC	CGCCATAAT
	bla							
3281	TCCCGTATTG	ACGCGGGCA	AGAGCAACTC	GGTCGCGCA	TACACTATTC	TCAGAATGAC	TTGGTTGAGT	ACTCACCAGT
	AGGGCATAAC	TGCGGCCCGT	TCTCGTTGAG	CCAGCGCGGT	ATGTGATAAG	AGTCTTACTG	AAACCACTCA	TGAGTGGTCA
	bla							
3361	CACAGAAAAG	CATCTTACGG	ATGGCATGAC	AGTAAGAGAA	TTATGCAGTG	CTGCCATAAC	CATGAGTGTAT	AAACTGCGG
	GTGCTTTTC	GTAGAATGCC	TACCGTACTG	TCATTCTCTT	AATACGTCAC	GACGGTATTG	GTACTACTA	TTGTAGCCG
	bla							
3441	CCAACCTACT	TCTGACAACG	ATCGGAGGAC	CGAAGGAGCT	AACCGTTTTT	TTGCACAACA	TGGGGGATCA	TGTAACTCGC
	GGTTGAATGA	AGACTGTGTC	TAGCCTCTG	GCTTCTCTGA	TTGGCGAAA	AACGTGTGTG	ACCCCTAGT	ACATTGAGCG

Fig. 13b

pEXHAM7/E4

	bla															
3521	CTTGATCGTT	GGGAACCGGA	GCTGAATGAA	GCCATACCAA	ACGACGAGCG	TGACACCACG	ATGCCTGTAG	CAATGGCAAC	GAAGTAGCAA	CCCTTGGCCT	CGACTTACTT	CGGTATGGTT	TGCTGCTCGC	ACTGTGGTGC	TACGGACATC	GTTACCGTTG
	bla															
3601	AACGTTGGCG	AAACTATTAA	CTGGCGAACT	ACTTACTCTA	GCTTCCCGGC	AACRAATTAAT	AGACTGGATG	GAGCGGGATA	TTGCAACGCG	TTTGATAAAT	GACCGCTTGA	TGAATGAGAT	CGAAGGGCCG	TTGTTAATTA	TCTGACCTAC	CTCCGCTTAT
	bla															
3681	AAGTTGCAGG	ACCACITCTG	CGCTCGGCC	TTCGGGCTGG	CTGGTTTATT	GCTGATAAAT	CTGGAGCCGG	TGAGCGTGGG	TTCAACGTCC	TGGTGAAGAC	GCGAGCCGGG	AAGGCCGACC	GACCAAATAA	CGACTATTTA	GACCTCGGCC	ACTCGCACCC
	bla															
3761	TCTCGCGGTA	TCATTCGAGC	ACTGGGGCCA	GATGGTAAGC	CCTCCCSTAT	CGTAGTTATC	TACACGACGG	GGAGTCAGGC	AGAGCGCCAT	AGTAACGTGC	TGACCCCGGT	CTACCATTCG	GGAGGGCATA	GCATCAATAG	ATGTGCTGCC	CCTCAGTCCG
	bla															
3841	AACATATGGAT	GAACGAAATA	GACAGATCGC	TGAGATAGGT	GCCTCACTGA	TTAAGCATTG	GTAAGTGTCA	GACCAAGTTT	TTGATACCTA	CTTGCTTTAT	CTGTCTAGCG	ACTCTATCCA	CGGAGTGACT	AATTCGTAAC	CATTGACAGT	CTGGTTCAAA
3921	ACTCATATAT	ACTTTAGATT	GATTTAAAC	TTCATTTTTA	ATTTAAAGG	ATCTAGGTGA	AGATCCTTTT	TGATAATCTC	TGAGTATATA	TGAAATCTAA	CTAAATTTTG	AAGTAAAAAT	TAATTTTTCC	TAGATCCACT	TCTAGGAAAA	ACTATTAGAG
4001	ATGACCAAAA	TCCCTTAAAG	TGAGTTTTCG	TTCCTGAG	CGTCAGACCC	CGTAGAAAAG	ATCAAAGGAT	CTTCTTGAGA	TACTGGTTTT	AGGGAATTGC	ACTCAAAGC	AAGGTGACTC	GCAGTCTGGG	GCATCTTTTC	TAGTTTCCTA	GAAGAACTCT
4081	TCCTTTTTTT	CTGCGGTAA	TCTGCTGCTT	GCAAAACAAA	AAACCACCGC	TACCAGCGGT	GGTTTGTTG	CCGGATCAAG	AGGAAAAAAA	GACGCGCATT	AGACGACGAA	CGTTTGTITT	TTTGGTGGCG	ATGGTCCGCA	CCAAACAAAC	GGCCTAGTTC
4161	AGCTACCAAC	TCTTTTTCCG	AAGGTAAGTG	GCTTCAGCAG	AGCGCAGATA	CCAAATACTG	TCCTTCTAGT	GTAGCCGTAG	TCGATGGTTG	AGAAAAAGGC	TTCCATTGAC	CGAAGTCTGC	TCGCGTCTAT	GGTTTATGAC	AGGAAAGATCA	CATCGGCATC
4241	TTAGGCCACC	ACTTCAAGAA	CTCTGTAGCA	CCGCCTACAT	ACCTCGCTCT	GCTAATCCTG	TTACCAGTGG	CTGCTGCCAG	AATCCGGTGG	TGAAGTCTCT	GAGACATCGT	GGCGGATGTA	TGGAGCGAGA	CGATTAGGAC	AATGGTCACC	GACGACGGTC
4321	TGGCGATAAG	TCTGTCTTA	CCGGGTGGA	CTCAAGACGA	TAGTTACCGG	ATAAGGCGCA	GCGGTGGGG	TGAACGGGGG	ACCGCTATTG	AGCACAGAAAT	GGCCCAACCT	GAGTCTGCT	ATCAATGGCC	TATTCGCGT	CGCCAGCCCG	ACTTCCCCCC
4401	GTTCTGTGAC	ACAGCCGAGC	TTGGAGCGAA	CGACCTACAC	CGAACTGAGA	TACCTACAGC	GTGAGCTATG	AGAAAGCGCC	CAAGCACGTC	TGTGCGGTGC	AACCTCGCTT	GCTGGATGTC	GCTTGACTCT	ATGGATGTCG	CATTCGATAC	TCITTCCGCG
4481	ACGCTTCCCG	AAGGGAGAAA	GGCGGACAGG	TATCCGGTAA	GCGGCAGGGT	CGGAACGGA	GAGCGCACGA	GGGAGCTTCC	TGCGAAGGGC	TTCCCTCTTT	CCGCCGTGCC	ATAGGCCAAT	CGCCGTCCCA	GCCTTGTCTC	CTCGCGTGTCT	CCCTCGAAGG
4561	AGGGGGAAAC	GCCTGTATC	TTTATAGTCC	TGTGCGGTTT	CGCCACTCT	GACTTGAGCG	TGATTTTTG	TGATGTCTGT	TCCCCCTTTG	CGGACCATAG	AAATATCAGG	ACAGCCCAAA	GCGGTGAGGA	CTGAACTCGC	AGCTAAAAAC	ACTACGAGCA
4641	CAGGGGGGCG	GAGCCTATGG	AAAAACGCCA	GCAACGCGGC	CTTTTACGG	TTCTGGCCT	TTTGTGGCC	TTTTGCTCAC	GTCCCCCGCG	CTCGGATACC	TTTTGCGGT	CGTTGCGCCG	GAAAAATGCC	AAGGACCGGA	AAACGACCGG	AAAAACGAGTG
4721	ATGTTCTTTC	CTGCGTTATC	CCCTGATTCT	GTGATAACC	GTATTACGCG	CTTGTAGTGA	GCTGATACCG	CTCGCGCAG	TACAAGAAAG	GAGGCAATAG	GGGACTAAGA	CACCTATTGG	CATAATGGCG	GAAACTCACT	CGACTATGGC	GAGCGGCGTC
4801	CCGAACGACC	GAGCGCAGCG	AGTCAGTGAG	CGAGGAAGCG	GAAGAGCGCC	CAATACGCAA	ACCGCCTCTC	CCCGCGCGTT	GGCTTGTGG	CTCGCGTCCG	TCAGTCACTC	GCTCCTCCG	CTTCTCGCGG	GTTATGCGTT	TGGCGGAGAG	GGCGCGCAA
4881	GGCCGATTCA	TTAATGCAGG	TATCAGGAG	CCCTTTCGTC	CTCAC				CCGGTAAGT	AATTACGTCC	ATAGTCTCC	GGJAAAGCAG	GAGTG			

pEXHAM7/C9

CAP-binding site -35 (lac)

1 CTCGAGAGCG GGCAGTGAGC GCAACGCAAT TAATGTGAGT TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC  
 GAGCTCTCGC CGTCACTCG CGTTGCGTTA ATTACTCA ATCGAGTGAG TAATCCGTGG GGTCCGAAAT GTGAAATACG

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-10 (lac) lac-operator peIB-leader

81 TTCGGCTCG TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTACACA GAATTCATTA AAGAGGAGAA ATTAACCATG  
 AAGGCCGAGC ATACAACACA CCTAACACT CGCTATTGT TAAAGTGTGT CTTAAGTAAT TTCTCCTTT TAATTGGTAC

---

peIB-leader

161 AAATACCTAT TGCCTACGGC AGCCGCTGSC TTGCTGCTGC TGGCAGCTCA GCCGGCCATG GCGCAGGTAC AGCTGCAGGA  
 TTTATGGATA ACGGATGCCG TCGGCGACCG AACGACGACG ACCGTGAGT CCGCCGGTAC CGCGTCCATG TCGACGTCCT

---

NcoI

241 GTCTGGGGGA GCGCTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCCTCTGG ATTCTCCTTC AGTAATTATG  
 CAGACCCCTT CCGCACCAGG TCGGACCTTC CAGGGACTCT GAGAGGACAC GTCGGAGACC TAAGAGGAAG TCATTAAATC

---

HCDR1 HCDR2

321 GCATACACTG GGTCCGCGAG GCTCCAGGCA AGGGCTGGA GTGGGTGGCA CTTATATCAT ATGATGGAAA TAAGAAATTC  
 CGTATGTGAC CCAGGCGGTC CGAGGTCGCT TCCCGACCT CACCCACCGT GAATATAGTA TACTACCTTT ATTCTTTAAG

---

HCDR2

401 TATGCAGACT CCGTGAAGGG CCGATTGCC ATCTCCAGAG ACACTTCTAA GAATACGGTG GATCTGCAAA TGACCAGCCT  
 ATACGTCTGA GGCACCTCCC GGTAAGCGG TAGAGTCTC TGTGAAGAIT CTTATGCCAC CTAGACGTTT ACTGGTGCCT

---

stuffer

481 GAGACCTGAG GACACGTCTT CAGCGCTGAG CTCGAAGACT GGTCAACGTC TCCTCAGCCT CCACCAAGGG CCCAAAGCTT  
 CTCGTGACTC CTGTGCAGAA GTCGCGACTC GAGCTTCTGA CCAGTGGCAG AGGAGTCGGA GGTGGTTCCC GGGTTTGGAA

---

BbsI HindIII

C9-light chain (lambda)

YOL-linker MnlI

561 GAAGAAGGTG AATTTTCAGA AGCACGCGTA TCCTATGAAC TGAATCAGCC ACCCTCGGTG TCAGTGGCCC CAGGACAGAC  
 CTCTCTCCAC TTAAGAGTCT TCGTGGCAT AGGATACTTG ACTGAGTCGG TGGAGGCCAC AGTCACCGGG GTCCTGTCTG

---

LCDR1

641 GGCCATGATT ACCTGTGGGG GAAACAACAT TGGAAAGTACA ACCGTGCACT GGTATCAGCA GAAGCCAGGC CAGGCCCTCG  
 CCGGTACTAA TGGACACCCC CTTTGTGTGA ACCTTCATGT TGGCACGTGA CCATAGTCGT CTTCGGTCCG GTCGGGGGAC

---

LCDR2

C9-light chain (lambda)

721 TGCTGGTGGT CTATGATGAT AACGAGCGAC CCTCAGGGAT CCTGAGCGA TTCTCTGGCT CCACTCTGG GAGCACGGCC  
 ACGACAGCA GATACTACTA TTGCTCGCTG GAGATCCCTA GGGACTCGCT AAGAGACCGA GGTGAGAGC CTCGTGCCGG

---

LCDR3

C9-light chain (lambda)

801 ACCCTGACCA TCAACAGGGT CGAAGCGGG GATGAGGCCG ACTATTATTG TCAAGTGTGG GATAGTGGTA GTGATCATGT  
 TGGACTCGT AGTGTCCCA GCTTCGGCCC CTACTCGGCG TGATAATAAC AGTTCACACC CTATACCAT CACTAGTACA

---

LCDR3

C9-light chain (lambda)

881 GGTATTCCGC GGAGGGACGA AGCTGACCGT CCTAGGTCAG CCAAAGGCTG CCCCCTCGGT CACTCTGTTT CCGCCCTCTC  
 CCATAAGCCG CCTCCCTGCT TCGACTGGCA GGTATCCAGT GGGTTCGCG GGGGGAGCCA GTGAGACAAG GCGGGGAGGA

---

NotI 6xHis amber<sup>r-s1</sup> c-myc

961 CTGCGGCCGC TGGATCCCAT CACCATCAC ATCACTAGGA ACAAAAGCTG ATCTCAGAAG AGGACCTAAA CGGATCCAAA  
 GACGCCGCGC ACCTAGGGTA GTGGTAGTGG TAGTGATCCT TGTTTTCGAC TAGAGCTTC TCCTGGATTT GCCTAGTTTT

---

pIII

1041 GATATCAGAG CTGAAACTGT TGAAGTGTG TTAGCAAAAT CCCATACAGA AAATTCATTT ACTAACGTCT GGAAGACGA  
 CTATAGTCTC GACTTTGACA ACTTTCAACA AATGTTTTTA GGGTATGCT TTTAAGTAAA TGATTGCAGA CCTTCTGCT

---

pIII

1121 CAAAACCTTA GATCGTTACG CTAACATGA GGGCTGCTG TGAATGCTA CAGGCGTGT AGTTTGTACT GGTGACGAAA  
 GTTTTGAAT CTAGCAATGC GATTGATACT CCCGACAGAC ACCTTACGAT GTCCGCAACA TCAAACATGA CCACTGCTTT

---

pIII

1201 CTCAGTGTFA CGGTACATGG GTTCCTATTG GGCTTGCTAT CCTGAAAAT GAGGGTGGTG GCTCTGAGGG TGGGGTCTT  
 GAGTCACAAT GCCATGTACC CAAGDATAAC CCGAACGATA GGGACTTTTA CTCCACACAC CGAGACTCCC ACCGCCAAGA

---

pIII

1281 GAGGGTGGCG GTTCTGAGGG TGGCGTACT AAACCTCCTG AGTACGGTGA TACACCTATT CCGGCTATA CTTATATCAA  
 CTCCACCGC CAAGACTCCC ACCGCCATGA TTTGGAGGAC TCATGCCACT ATGTGGATAA GGCCCGATAT GAATATAGTT

---

pIII

1361 CCCTCTCGAC GGCACCTTATC CGCCTGGTAC TGAGCAAAAC CCGCTAATC CTAATCCTTC TCTTGAGGAG TCTCAGCCTC  
 GGGAGAGCTG CCGTGAATAG GCGGACCATG ACTGTTTTG GGGCGATTAG GATTAGGAAG AGAACTCCTC AGAGTCGGAG

---

pIII

1441 TTAATACTTT CATGTTTCAG AATAATAGGT TCCGAAATAG GCAGGGGGCA TTAACTGTTT ATACGGGCAC TGTTACTCAA  
 AATTATGAAA GTACAAAGTC TTATTATCCA AGGCTTTATC CGTCCCCGCT AATTGACAAA TATGCCCGTG ACAATGAGTT

Fig. 14a

pEXHAM7/C9

	pIII															
1521	GGCACTGACC	CCGTAAAAAC	TTATTACCAG	TACACTCCTG	TATCATCAAA	AGCCATGTAT	GACGCTTACT	GGAAACGGTAA	CCGTGACTGG	GGCAATTTTG	AATAATGGTC	ATGTGAGGAC	ATAGTAGTIT	TCGGTACATA	CTGCGAATGA	CCTTGCCATT
	pIII															
1601	ATTTCAGAGAC	TGCGCTTTCC	ATTCTGGCCT	TAATGAGGAT	TTATTTGTTT	GTGAATATCA	AGGCCAATCG	TCTGACCTGC	TAAGTCTCTG	ACCGGAAAAG	TAAGACCGAA	ATTACTCTTA	ATAAACAACA	CACCTATAGT	TCCGGTTAGC	AGACTGGAGC
	pIII															
1681	CTCAACCTCC	TGTCATAGCT	GGCGGCGGCT	CTGGTGGTGG	TTCTGGTGGC	GGCTCTGAGG	GTGGTGGCTC	TGAGGGTGGC	GAGTTGGAGG	ACAGTTACGA	CCGCCGCCGA	GACCACCACC	AAGACCACC	CCGAGACTCC	CACCACCGAG	ACTCCCACC
	pIII															
1761	GGTCTGAGG	GTGGCGGCTC	TGAGGGAGGC	GGTCCCGTGG	GTGGCTCTGG	TTCCGGTGGT	TTTGATTATG	AAAAGATGGC	CCAAGACTCC	CACCGCCGAG	ACTCCCTCCG	CCAAGGCCAC	CACCGAGACC	AAGGCCACTA	AAACTAATAC	TTTTCTACCG
	pIII															
1841	AAACGCTAAT	AAGGGGGCTA	TGACCGAAAA	TGCCGATGAA	AACGGCTAC	AGTCTGACGC	TAAAGGCCAA	CTTGATCTCG	TTTGCGAITA	TTCCCCCGAT	ACTGGCTTTT	ACGGCTACTT	TTGCGCGATG	TCAGACTGCG	ATTTCCGTTT	GAACAAAGAC
	pIII															
1921	TCGCTACTGA	TTACCGTGCT	GCTATCGATG	GTTCATTGG	TGACGTTTCC	GGCCTTGCTA	ATGGTAAATG	TGCTACTGGT	AGCGATGACT	AATGCCACGA	CGATAGCTAC	CAAAATAACC	ACTGCAAAAGG	CCGGAAACGAT	TACCATTACC	ACGATGACCA
	pIII															
2001	GATTTTGCTG	GCTCTAATTC	CCAAATGGCT	CAAGTCGGTG	ACGGTGATAA	TTCACCTTTA	ATGAATAATT	TCCGTCATAA	CTAAAACGAC	CGAGATTAAG	GGTTTACCGA	GTTCAGCCAC	TGCCACTATT	AAGTGGAAAT	TACTTATTA	AGGCAGTTAT
	pIII															
2081	TTTACCTTCC	CTCCCTCAAT	CGGTTGAATG	TCGCCCTTIT	GTCTTTGGCG	CTGGTAAACC	ATATGAATTT	TCTATTGATT	AAATGGAAGG	GAGGGAGITA	GCCAACTTAC	AGCGGGAAAA	CAGAAAACCG	GACCATTGG	TATACTTAA	AGATAACTAA
	pIII															
2161	GTGACAAAAT	AAACTTATTC	CGTGGTGCT	TTGCGTTTCT	TTTATATGIT	GCCACCTTTA	TGTATGTATT	TTCTACGTTT	CACGTGTTTA	TTGAATAAG	GCACCACAGA	AACGCAARA	AAATATACAA	CGGTGGAART	ACATACATA	AAGATGCAAA
	pIII															
2241	GCTAACATAC	TGCGTAATAA	GGAGTCTTAA	TGATCTAGAG	GCCTGTGCTA	ATGATCAGCT	AGCTTGAGGC	ATCAATAAAA	CGATTGTATG	ACGCATTATT	CCTCAGAATT	ACTAGATCTC	CGGACACGAT	TACTAGTCGA	TCGAACTCCG	TAGTTATTTT
2321	CGAAAGGCTC	AGTCGAAAGA	CTGGGCTTTT	CGTTTTATCT	GTGTGTTGTC	GGTTAACGTC	GACCTGGCGT	AATAGCGAAG	GCTTTCCGAG	TCAGCTTCT	GACCCGGAAA	GCAAAATAGA	CAACAAACAG	CCAATTGCAG	CTGGACCGCA	TTATCGCTTC
2401	AGGCCCGCAC	CGATCGCCCT	TCCCAACAGT	TGCGCAGCCT	GAATGGCGAA	TGGGACGCGC	CCTGTAGCGG	CGCATTAAGC	TCCGGGCGTG	GCTAGCGGGA	AGGGTTGTC	ACGCGTCGGA	CTTACCGCTT	ACCTGCGCG	GGACATCGCC	CGGTAATTCG
2481	CGCGCGGGTG	TGGTGGTTAC	GCGCAGCGTG	ACCGCTACAC	TTGCCAGCGC	CCTAGCGCCC	GCTCCTTTCG	CTTCTTCC	CGCGCCCGAC	ACCACCAATG	CGCGTCGCAC	TGGCGATGTG	AACGGTCCGC	GGATCGCGGG	CGAGGAAAGC	GAAAGAGGG
2561	TTCTTTTCT	GCCACGTTCC	CGGGCTTCC	CCGTCAGCT	CTAAATCGGG	GGCTCCCTTT	AGGGTCCCGA	TTAGTGCTT	AAGGAAAGAG	CGGTGCAAGC	GGCCGAAAGG	GGCAGTTCTGA	GATTTAGCCC	CCGAGGGAAA	TCCCAAGGCT	AAATCAGGAA
2641	TACGGCACCT	CGACCCCAA	AAACTTGATT	AGGGTATGG	TTACAGTAGT	GGGCCATCGC	CCTGATAGAC	GGTTTTCCG	ATGCCGTGGA	GCTGGGTTT	TTTGAACATA	TCCCACTACC	AAGTGCATCA	CCCGGTAGCG	GGACTATCTG	CCAAAAGCG
2721	CCTTTGACGT	TGGAGTCCAC	GTTCTTAAAT	AGTGGACTCT	TGTTCCAAAC	TGGAAACAAC	CTCAACCCTA	TCTCGGTTA	GGAAACTGCA	ACCTCAGGTG	CAAGAAATA	TCACCTGAGA	ACAAGTTTG	ACCTTGTGTG	GAGTTGGGAT	AGAGCCAGAT
2801	TTCTTTGAT	TTATAAGGGA	TTTTGCCGAT	TTCCGCTTAT	TGGTTAAAA	ATGAGCTGAT	TTAACAAAA	TTTAAACCGA	AAGAAAACATA	AATATTTCC	AAAACGGCTA	AAGCCGGATA	ACCAATTTT	TACTCGGATA	AATGTTTTT	AAATGCGCT
2881	ATTTTAAACA	AATATTAACG	CTTACATTT	AGGTGGCACT	TTTCGGGGAA	ATGTGCGCGG	AACCCCTATT	TGTTTTATTT	TAAAATTGTT	TTATAATTGC	GAATGTTAAA	TCCACCGTGA	AAAGCCCTT	TACACGCGCC	TTGGGGATA	ACAAATAAAA
2961	TCTAAATACA	TTCAAAATG	TATCCGCTCA	TGAGACAATA	ACCCTGATA	ATGCTTCAAT	AATATTGAAA	AAGGAAGAGT	AGATTTATGT	AAGTTTATAC	ATAGCCGAGT	ACTCTGTTAT	TGGACTATT	TACGAAGTTA	TTATACTTT	TTCTTCTCA
	bla															
3041	ATGAGTATTC	AACATTTCCG	TGTCGCCCTT	ATTCCCTTTT	TTGCGGCATT	TTGCCCTTCT	GTTTTTGCTC	ACCCAGAAAC	TACTCATAAG	TGTAAAGGC	ACAGCGGGAA	TAAGGGAAAA	AACGCCGTAA	AACGGAAGGA	CAAAAACGAG	TGGGTCTTTG
	bla															
3121	GCTGGTGAAA	GTAAGAGATG	CTGAAGATCA	GTTGGGTGCA	CGAGTGGGTT	ACATCGAACT	GGATCTCAAC	AGCCGTAAGA	CGACCACCTT	CATTTTCTAC	GACTTCTAGT	CAACCCACGT	GCTCACCCAA	TGTAGCTTGA	CCTAGAGTTG	TCGCATTCT
	bla															
3201	TCCTTGAGAG	TTTTCGCCCC	GAAGAAGCTT	TTCCAATGAT	GAGCACTTTT	AAAGTTCTGC	TATGTGGCGC	GGTATTATCC	AGAACTCTC	AAAAGCGGGG	CTTCTTGCAA	AAGGTTACTA	CTCGTAAAA	TTCAAGACG	ATACACCGCG	CCATAATAGG
	bla															
3281	CGTATTGACG	CCGGGCAAGA	GCAACTCGGT	CGCCGCATAC	ACTATTCTCA	GAATGACTTG	GTTGAGTACT	CACCACTCAC	GCATAACTGC	GGCCCGTTCT	CGTTGAGCCA	GCGCGTATG	TGATAAGAGT	CTTACTGAAC	CAACTCATGA	GTGGTCAGTG
	bla															
3361	AGAAAAGCAT	CTTACGGATG	GCATGACAGT	AAGAGAATTA	TGCAGTGTCT	CCATAACCAT	GAGTGATAAC	ACTGCGCCA	TCTTTTGGTA	GAATGCCTAC	CGTACTGTCA	TTCTCTTAAT	ACGTCAACGAC	GGTATTGGTA	CTCACTATTG	TGACGCGGTT
	bla															
3441	ACTTACTTCT	GACAACGATC	GGAGGACCGA	AGGAGCTAAC	CGCTTTTITG	CACAACATGG	GGGATCATGT	AACTCGCCTT	TGAATGAAGA	CTGTTGCTAG	CCTCCTGGCT	TCTCTGATTG	GCGAAAAAAC	GTGTTGTACC	CCCTAGTACA	TTGAGCGGAA

Fig. 14b.

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	bla															
3521	GATCGTTGGG	AACCGGAGCT	GAATGARGCC	ATACCAAACG	ACGAGCGTGA	CACCACGATG	CCTGTAGCAA	TGGCAACAAC	CTAGCAACCC	TTGGCCTCGA	CTTACTTCGG	TATGSTTTGC	TGCTCGCACT	GTGGTGCTAC	GGACATCGTT	ACCGTTGTTG
	bla															
3601	GTTGCGCAAA	CTATTAACCTG	GCGAACTACT	TACTCTAGCT	TCCCGGCAAC	AATTAATAGA	CTGGATGGAG	GCGGATAAAG	CAACGCGTTT	GATAATTGAC	CGCTTGATGA	ATGAGATCGA	AGGGCCGTTG	TTAATTATCT	GACCTACCTC	CGCCTATTTC
	bla															
3681	TTGCAGGACC	ACTTCTGCGC	TGGGCCCTTC	CGGCTGGCTG	GTTTATGCT	GATAAACTG	GAGCCGGTGA	GCGTGGGTCT	AACGTCCTGG	TGAAGACGCG	AGCCGGGAAG	GCCGACCCGAC	CAAATAACGA	CTATTTAGAC	CTCGGCCACT	CGCACCCAGA
	bla															
3761	CGCGGTATCA	TTGCAGCACT	GGGGCCAGAT	GGTAAGCCCT	CCCGTATCGT	AGTTATCTAC	ACGACGGGGA	GTCAGGCAAC	GCGCCATAGT	AACGTCGTGA	CCCGGCTCTA	CCATTCCGGA	GGGCATAGCA	TCAATAGATG	TGCTGCCCTT	CAGTCCGTTG
	bla															
3841	TATGGATGAA	CGAAATAGAC	AGATCGCTGA	GATAGGTGCC	TCACTGATTA	AGCATTGGTA	ACTGTCAGAC	CAAGTTTACT	ATACCTACTT	GCTTTATCTG	TCTAGCGACT	CTATCCACGG	AGTGACTAAT	TCGTAACCAT	TGACAGTCTG	GTTCAAATGA
3921	CATATATACT	TTAGATTGAT	TTAAAACCTC	ATTTTAAATT	TAAAAGGATC	TAGGTGAAGA	TCCTTTTGA	TAATCTCATG	GTATATATGA	AATCTAACTA	AATTTTGAAG	TAAAAATTAA	ATTTTCTAG	ATCCACTTCT	AGGAAAAACT	ATTAGAGTAC
4001	ACCAAAATCC	CTTAACGTGA	GTTTTCGTTC	CACTGAGCCT	CAGACCCCGT	AGAAAAGATC	AAAGGATCTT	CTTGAGATCC	TGGTTTTAGG	GAATTGCACT	CAAAAGCAAAG	GTGACTCGCA	GTCTGGGGCA	TCTTTTCTAG	TTTCTAGAA	GAACTCTAGG
4081	TTTTTTTCTG	CGCGTAATCT	GCTGCTTGCA	AACAAAAAAA	CCACCGTAC	CAGCGGTGGT	TTGTTTGGCG	GATCAAGAGC	AAAAAAGAC	GCGCATTAGA	CGACGAACGT	TTGTTTTTTT	GGTGGCGATG	GTCCGCCACCA	AACAAACGGC	CTAGTTCTCG
4161	TACCAACTCT	TTTTCCGAAG	GTAACCTGGT	TCAGCAGAGC	GCAGATACCA	AATACTGTCC	TCTAGTGTGA	GCCGTAGTTA	ATGTTGAGA	AAAAGGCTTC	CATTGACCGA	AGTCGTCTCG	CGTCTATGGT	TTATGACAGG	AAGATCACAT	CGGCATCAAT
4241	GGCCACCCT	TCAAGAACTC	TGTAGCACCG	CCTACATACC	TCCGCTGTCT	AATCCGTGTA	CCAGTGGCTG	CTGCCAGTGG	CCGGTGGTGA	AGTCTTTGAG	ACATCGTGGC	GGATGTATGG	AGCGAGACGA	TTAGGACAAT	GGTCACCGAC	GACGGTCAAC
4321	CGATAAGTCG	TGTCTTACCG	GTTTGGACTC	AAGACGATAG	TTACCGGATA	AGGCGCAGCG	GTCGGGCTGA	ACGGGGGGTT	GCTATTACAG	ACAGAATGGC	CCAACCTGAG	TTCTGCTATC	AATGGCCTAT	TCCGCGTCCG	CAGCCCGACT	TGCCCCCAA
4401	CGTGACACACA	GCCCAGCTTG	GAGCGAACGA	CCTACACCGA	ACTGAGATAC	CTACAGCGTG	AGCTATGAGA	AAGCGCCACG	GCACGTGTGT	CGGGTCGAAC	CTCGCTTGCT	GGATGTGGCT	TGACTCTATG	GATGTCCGAC	TCGATACTCT	TTCCGGGTGC
4481	CTTCCGAAAG	GGAGAAAGGC	GGACAGGTAT	CCGGTAAGCG	GCAGGGTCCG	AACAGGAGAG	CGCACGAGGG	AGCTTCCAGG	GAAGGGCTTC	CCTCTTTCCG	CCTGTCCATA	GGCCATTCCG	CGTCCAGCC	TTGTCTCTC	CGGTCTCCC	TCGAAGGTCC
4561	GGGAAACGCC	TGGTATCTTT	ATAGTCCCTGT	CGGGTTTCGC	CACTCTGAC	TTGAGCGTGG	ATTTTGTGA	TGCTCTGTCG	CCCTTTGCGG	ACCATAGAAA	TATCAGGACA	GCCCAAAGCG	GTGGAGACTG	AACTCGCAGC	TAAAAACT	ACGAGCAGTC
4641	GGGGGCGGAG	CCTATGGAAA	AACGCCAGCA	ACGCGGCCCT	TTTACGGTTC	CTGGCCTTTT	GCTGGCCTTT	TGCTCACATG	CCCCCGCTC	GGATACCTTT	TTGCGGTCTG	TGCGCCGAAA	AAATGCCAAG	GACCCGAAAA	CGACCCGAAA	ACGAGTGTAC
4721	TTCTTTCTCG	CGTTATCCCC	TGATTCTGTG	GATAACCGTA	TTACCGCCTT	TGAGTGAGCT	GATACCGCTC	GCCGACGCCG	AAGAAAGGAC	GCAATAGGGG	ACTAAGACAC	CTATTGGCAT	AATGGCGGAA	ACTCACTCGA	CTATGGCGAG	CGGGCTCGGC
4801	AACGACCGAG	CGCAGCGAGT	CAGTGAGCGA	GGAAGCGGAA	GAGCGCCCAA	TACGCAAACC	GCCTCTCCCC	GCGCGTTGGC	TTGCTGGCTC	GCGTCTCTCA	GTCACTCGCT	CCTTCCGCTT	CTCGCGGTTT	ATGCGTTTGG	CGGAGAGGGG	CGCGCAACCG
4881	CGATTCATTA	ATGCAGGTAT	CACGAGGCC	TTTCGTCTC	AC				GCTAAGTAAT	TACGTCCATA	GTGCTCCGGG	AAAGCAGGAG	TG			

Fig. 14c

**REFERENCES CITED IN THE DESCRIPTION**

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

**Patent documents cited in the description**

- WO 8909622 A [0015]
- WO 8901783 A [0015]
- EP 0239400 A [0015]
- WO 9007861 A [0015]
- EP 01123851 A [0074]

**Non-patent literature cited in the description**

- **SHATILL et al.** *J. Biol. Chem.*, 1985, vol. 260 (20), 11107-11114 [0002]
- **COLLER B. et al.** *J. Clin. Invest.*, 1983, vol. 72, 325-338 [0003]
- **BHATT DL ; TOPOL EJ.** *JAMA*, 2000, vol. 284 (12), 1549-58 [0003]
- **TOPOL EJ et al.** *Lancet*, 1999, vol. 353 (9148), 227-31 [0003]
- **DASGUPTA H. et al.** *Am Heart J*, 2000, vol. 140 (2), 206-11 [0003]
- **HILLEGASS WB et al.** *Pharmacoeconomics*, 2001, vol. 19 (1), 41-55 [0003]
- **CHEW DP. et al.** *Circulation*, 2001, vol. 103 (2), 201-206 [0003]
- **PETER K. et al.** *Blood*, 1998, vol. 92 (9), 3290 [0003]
- **GAWAZ M.** *Therapie bei koronarer Herzerkrankung*, 1999 [0003]
- **COLCHER et al.** *Cancer Research*, 1989, vol. 49, 1732-1745 [0015]
- **SAMBROOK et al.** *Molecular Cloning - A Laboratory Manual*. Cold Spring Harbor, 1989 [0015] [0019]
- **HOLMGREN.** *Annual Rev. Biochem.*, 1985, vol. 54, 237 [0021]
- **LA VALLE et al.** *Bio/Technology*, 1993, vol. 11, 187 [0021]
- **WONG.** *Curr. Opin. Biotech.*, 1995, vol. 6, 517 [0021]
- **DAVIES.** *Curr. Opin. Biotech*, 1995, vol. 6, 543 [0021]
- **MANNINO et al.** *Biotechniques*, 1988, vol. 6, 682 [0034]
- **PETER et al.** *Blood*, 1998, vol. 9, 3240-3249 [0049]
- **PETER K ; O'TOOLE TE.** *J Exp Med.*, 1995, vol. 181 (1), 315-326 [0050]
- **LITTLE, M. et al.** *J. Immunol. Methods*, 1999, vol. 231, 3-9 [0062]

专利名称(译)	用于抑制血小板聚集的人源抗体		
公开(公告)号	<a href="#">EP1300419B1</a>	公开(公告)日	2007-06-13
申请号	EP2001123851	申请日	2001-10-05
申请(专利权)人(译)	AFFIMED THERAPEUTICS AG		
当前申请(专利权)人(译)	AFFIMED THERAPEUTICS AG		
[标]发明人	BUTTNER CLAUDIA SCHWARZ MEIKE KNACKMUSS STEFAN PETER KARLHEINZ ROTTGEN PETER LITTLE MELVYN		
发明人	BÜTTNER, CLAUDIA SCHWARZ MEIKE KNACKMUSS STEFAN PETER, KARLHEINZ RÖTTGEN PETER LITTLE, MELVYN		
IPC分类号	C07K16/28 A61K39/395 G01N33/53 C12N15/13 C12N5/10 C12N15/63 C12N15/09 A61K31/7088 A61K48/00 A61P7/02 A61P37/02 C12N11/15 C12N11/19 C12N1/21 C12P21/08		
CPC分类号	A61K2039/505 A61P7/02 A61P37/02 C07K16/2848 C07K2317/21 C07K2317/622		
其他公开文献	EP1300419A1		
外部链接	<a href="#">Espacenet</a>		

摘要(译)	ccatggcga agtcagctg gtgcagctg gagctgaggt gaataagcct gggcctcag 60
本发明涉及用于抑制血小板聚集的人源抗体或其衍生物，其特征在于它	tgaaggtctc ctgcaaggct tctggataca ccttcaccgg ctactatatg cactgggtgc 120
通过基本上排他性结合血小板整联蛋白受体GPIIb / IIIa的活化状态而有	gacaggcccc tggacaaggg cttgagtgga tgggatggat caaccctaac agtggtggca 180
效。	caactatgc acagaagttt cagggtctgg tcaccatgac cagggacacg tccatcagca 240
	ccgcctacat ggagctgagc aggtgagat ctgacgacac ggcctgtat tactgtgca 300
	gaggccgtgc tttgtataac cggaaagacc ggtccccaa ctggttegac ccctggggcc 360
	agggaaacct ggtcaccgtc tcctcaggga gtgcatccgc cccaacctt aagettgaag 420
	aaggtgaatt ttcagaagca cgcgtacagg ctgtgctgac tcagccgcc tcggtgtag 480
	tggccccagg acagacggcc aggtattact gtgggggaaa caacattgga agtaaaagtg 540
	tgcagtggtg ccagcagaag ccaggccagg cccctgtgct ggtcgtctat gatgatagcg 600
	accggccctc agggatccct gagcgattct ctggtccaa ctctgggaac atggccacc 660
	tgaccatcag cagggtcgaa gccgggatg aggcgacta ttactgtcag gtgtgggata 720
	gtagtagtga tcatgtggtg ttccggcgag ggaccaagct gaccgtccta ggtcagccca 780
	aggctgcccc ctcggtcaact ctgttccgc cgtccgcggc cgc 823