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(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

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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 administerd 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.

[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 specifity. 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 prbteinases. 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.

[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 phagedisplay library of natural or modified human antibody fragments, screened for antibodies with affinity for thrombocytes.

[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 immunoglobuline domains, e.g. via amino acid deletion, insertions, substitutions and/or recombinations. Methods for introducing such modifications in the coding sequence for the immunoglobuline 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.

[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.

[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.

[0018] A further preferred embodiment concers 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 regultatory 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 promotors are metallothein 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. cervisiae), insect cells (preferably sf9 cells) and animal cells (preferaly mammal cells). Prefered 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 libary 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.

[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.

[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, intra-arterially, intramuscularly, subcutaneously, intramedullarly, intrathecally, intraventricularly, intraveneously, 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 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:

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 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;

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

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 underligned

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 painted areas represent CDR regions; Oligonucleotides are represented by arrows and identified by numbers (c.f. Fig. 4). BpiI restriction endonuclease recognition sites are indicated.

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

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

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.

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

[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

[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

[0039] Total RNA is isolated from spleen samples of six human donors and peripheral blood lymphocytes (PBL) of five healthy human donors (app. 1-5x10^8 PBLs each, RNeasy (TM) Midiprep.Kit, Qiagen). From total RNA poly A^+-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

[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$^-$-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

[0041] Heavy chain fragments are digested with NcoI and HindIII, light chain fragments with *Mlu*I and *Not*I (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 *Nco*I and *Hind*III, 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 1.5x10^6 to 7.1x10^7 as determined by plating appropriate dilutions.

[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 *Mlu*I and *Not*I, purified by gel extraction and ligated

with lambda and kappa light chain fragments separately. Ligation is done in 20 μl volume using 1 μg 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 μl Tris 10mM, pH8.5. Of this 2 μl are used for transformation of 50 μl 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 $1.75x10^9$.

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 μg/ml ampicillin and 20 μg/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 μl 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), NaHCO3 (12 mM), KCl, MgCl (2 mM each), glucose, BSA (1mg/ml each), pH 7.4) containing 2% skimmed milk powder and incubated with $1.75x\ 10^{12}$ 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 transfered 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 $CaCl_2$, $MgCl_2$ (2 mM each), ADP (15 μM) 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 μl LB_{GAT} medium (LB medium containing 50 mM glucose, 100 μg/ml ampicillin and 20 μg/ml tetracyclin), plated on LB_{GAT} agarplates and incubated over night at 37 °C.

Packaging:

[0048] Colonies are scraped from agar plates using two times 5ml LB_{GAT} medium and used for inoculation of 20 ml LB_{GAT} medium at an OD600 of 0.1. Cells are incubated at 37 °C and 200 rpm for one hour and superinfected with app. $1x10^{10}$ M13K07 helperphages. After one hour at 37 °C cells are collected by centrifugation (5 min, 6000g) resuspended in LB medium supplemented with ampicillin (100μg/ml) and kanamycin (50 μg/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).

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

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

[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 $5x10^6$ 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 140 g and the supernatant cleared again by centrifugation (20 min, 3200 g).

Binding on activated GPIIb/IIIa:

[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. $4x10^6$ cells are incubated with 4 ml depleted phage solution for 30 min at room temperature.

Elution by antibody competition:

[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 µl 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:

[0052] Cells are centrifuged for 10 min at 140g, resuspended in 50 ml modified tyrode buffer (tyrode buffer pH 6 adjusted with Hepes, containing $CaCl_2$, $MgCl_2$ (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 µl Tris (2 M, pH 8) and cleared by centrifugation at 13000rpm for 10 min in a benchtop centrifuge.
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 manufactor (Quiagen). DNA is digested with *Bst*NI (New England Biolabs) and analysed on a 1% agarose gel.

Preparation of periplasmic extracts

[0054] 5 ml of LB_{GAT} medium are inoculated with 250 µl 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 µg/ml) and IPTG (100 µM) and incubated at 28 °C and 180 rpm over night. Cells are again harvested by centrifugation and resuspended in 500 µl 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

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

Analysis of activation specificity:

[0056] Complete citrate blood (S-Monovette, Sarstedt) is diluted 1/50 in 50 µl tyrode buffer with or without ADP (20 µM) and incubated for 20 min at room temperature with 10 µl 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:

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

Results

Selection of GPIIb/IIIa binding scFv's

[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*NI 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).

[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.

[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

[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

[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

*Bbs*I (*Bpi*I). Standard PCR reactions are set up using the oligonucleotid primers as shown in Fig. 4 to generate DNA fragments of the scFv regions 3'and 5' of the heavy chain CDR3 containing unique *Bpi*I 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). *Bpi*I restriction endonuclease recognition sites are indicated.

[0064] The stuffer DNA fragment is generated directly by hybridisation of synthetic oligonucleotides. DNA-fragements are cut with *Bpi*I and cloned in *Bpi*I 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 *Bbs*I necessitates the purification of two vector fragments, 3.8 and 0.5 kb in size. To avoid this both *Bbs*I 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 *Bbs*I 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 μM each) are filled in separately using oligonucleotides VHCDR3_for/cut and VHCDR3_back/cut (0.2 μM) (Fig. 4) with 1 unit Vent exo$^-$ DNA-polymerase (New England Biolabs) unter 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 μl Volume. PCR-products are purified using PCR purification Kit (Qiagen). 2/3 of the material is cut with 100 units *Bbs*I 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 *Bbs*I (1 unit/μg 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$^-$-DNA polymerase (New England Biolabs) and 0.2 μM primer in a volume of 100 μl 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 *Bbs*I, 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 $7.5x10^8$ 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 $7.5x10^{11}$ 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)

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 DLFRGRGDYGDYG 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).

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Lys Phe Gln Gly Trp Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr
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<210> 56
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<210> 59
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<210> 69
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<210> 75
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<400> 75
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<210> 76
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<400> 76
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<210> 77
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<400> 77
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<210> 78
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<400> 78
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<210> 79
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<400> 79

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<210> 80
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<400> 80
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<210> 81
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<400> 81
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<210> 82
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<400> 82
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<210> 83
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<400> 83
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<210> 84
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<400> 84
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<210> 85

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<210> 86
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<400> 86
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<400> 87
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<210> 89
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<210> 90
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<210> 93
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<210> 94
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<210> 97
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<210> 126
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1 5 10 15

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<212> PRT
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<220>
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<400> 129

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1 5 10 15

Thr Ser Phe Asp Tyr
20

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Phe Ala Phe Asp Ile
20

<210> 131
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<213> Artificial Sequence

<220>
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<210> 132
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1 5 10

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Tyr Tyr Gly Met Asp Val
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<400> 134

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Met Asp Val

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His Ala Phe Asp Ile
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<210> 139

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ggaaccctgg tcacc 75

<210> 143
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cagggaaccc tggtcacc                                                  78
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<210> 144
<211> 81
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<220>
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<210> 145
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide

<400> 145

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tggggccagg gaaccctggt cacc                                           84
```

Claims

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.

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).

5. Antibody according to claim 2 or 3, **characterised in that** the single chain antibody fragment is derived from an IgM or IgG antibody.

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.

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.
- 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 bond to said thrombocytes or other cells expressing active integrin receptor molecules.

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.

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.

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.

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

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.

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.

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

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.

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.

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

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 multivalentr 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,

- 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.

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

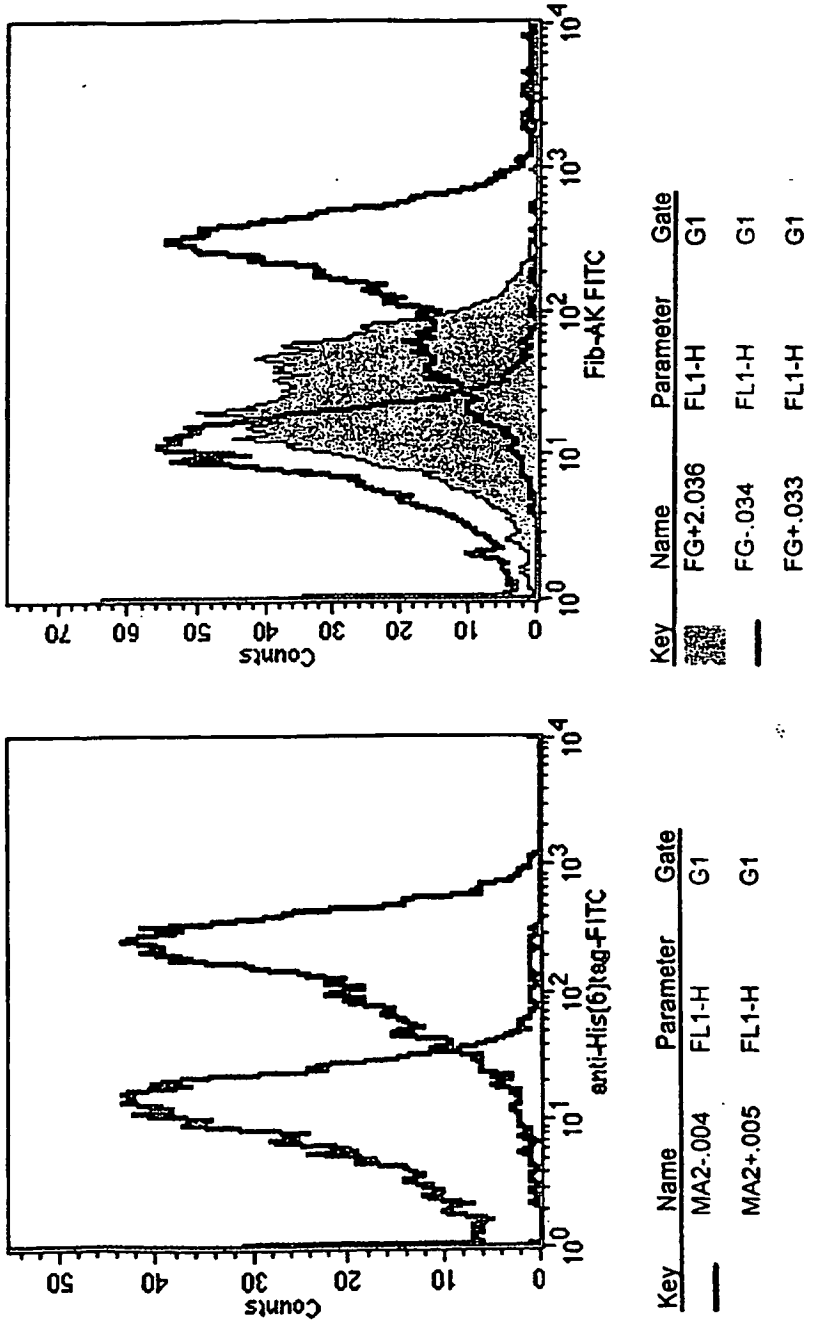
10. Vecteur d'expression contenant la séquence d'ADN selon la revendication 9.

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

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.

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.

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.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Fib-AK FITC
Key Name Parameter Gate
FG+2.036 FL1-H G1
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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 tgaaggtctc ctgcaaggct tctggataca ccttcaccgg ctactatatg cactgggtgc
121 gacaggcccc tggacaaggg cttgagtgga tgggatggat caaccctaac agtggtggca
181 caaactatgc acagaagttt cagggctggg tcaccatgac cagggacacg tccatcagca
241 ccgcctacat ggagctgagc aggctgagat ctgacgacac ggccgtgtat tactgtgcga
301 gaggccgtgc tttgtataac cggaacgacc ggtcccccaa ctggttcgac ccctggggcc
                                                          HindIII
361 agggaaccct ggtcaccgtc tcctcaggga gtgcatccgc cccaaccctt aagcttgaag
                          MluI
421 aaggtgaatt ttcagaagca cgcgtacagg ctgtgctgac tcagccgccc tcggtgtcag
481 tggccccagg acagacggcc aggattacct gtgggggaaa caacattgga agtaaaagtg
541 tgcagtggta ccagcagaag ccaggccagg cccctgtgct ggtcgtctat gatgatagcg
601 accggccctc agggatccct gagcgattct ctggctccaa ctctgggaac atggccaccc
661 tgaccatcag cagggtcgaa gccggggatg aggccgacta ttactgtcag gtgtgggata
721 gtagtagtga tcatgtggta ttcggcggag ggaccaagct gaccgtccta ggtcagccca
                                                    NotI
781 aggctgcccc ctcggtcact ctgttcccgc cgtccgcggc cgc
```

Fig. 2a

Translation of MB9

```
  1 MAEVQLVQSG AEVNKPGASV KVSCKASGYT FTGYYMHWVR QAPGQGLEWM
 51 GWINPNSGGT NYAQKFQGWV TMTRDTSIST AYMELSRLRS DDTAVYYCAR
101 GRALYNRNDR SPNWFDPWGQ GTLVTVSSGS ASAPTLKLEE GEFSEARVQA
151 VLTQPPSVSV APGQTARITC GGNNIGSKSV QWYQQKPGQA PVLVVYDDSD
201 RPSGIPERFS GSNSGNMATL TISRVEAGDE ADYYCQVWDS SSDHVVFGGG
251 TKLTVLGQPK AAPSVTLFPP SAAAGSHHHH HH*
```

Fig. 2b

C9 scFv: (Seq. No. 2)

*Nco*I
1 ccatggcgca ggtacagctg caggagtctg ggggaggcgt ggtccagcct gggaggtccc
61 tgagactctc ctgtgcagcc tctggattct ccttcagtaa ttatggcata cactgggtcc
121 gccaggctcc aggcaagggg ctggagtggg tggcacttat atcatatgat ggaaataaga
181 aattctatgc agactccgtg aagggccgat tcgccatctc cagagacact tctaagaata
241 cggtggatct gcaaatgacc agcctgagac ctgaggacac ggctgtatat tactgtgcga
301 aatctggggg tattgccttg tactgggggg aatttgacta ctggggccag ggaaccctgg
*Hind*III
361 tcaccgtctc ctcagcctcc accaagggcc caaagcttga agaaggtgaa ttttcagaag
*Mlu*I
421 cacgcgtatc ctatgaactg actcagccac cctcggtgtc agtggcccca ggacagacgg
481 ccatgattac ctgtgggggа aacaacattg gaagtacaac cgtgcactgg tatcagcaga
541 agccaggcca ggcccctgtg ctggtcgtct atgatgataa cgagcgaccc tcagggatcc
601 ctgagcgatt ctctggctcc aactctggga gcacggccac cctgaccatc aacagggtcg
661 aagccgggga tgaggccgac tattattgtc aagtgtggga tagtggtagt gatcatgtgg
721 tattcggcgg agggacgaag ctgaccgtcc taggtcagcc caaggctgcc ccctcggtca
*Not*I
781 ctctgttccc gccctcctct gcggccgc

E4 scFv: (Seq. No. 3)

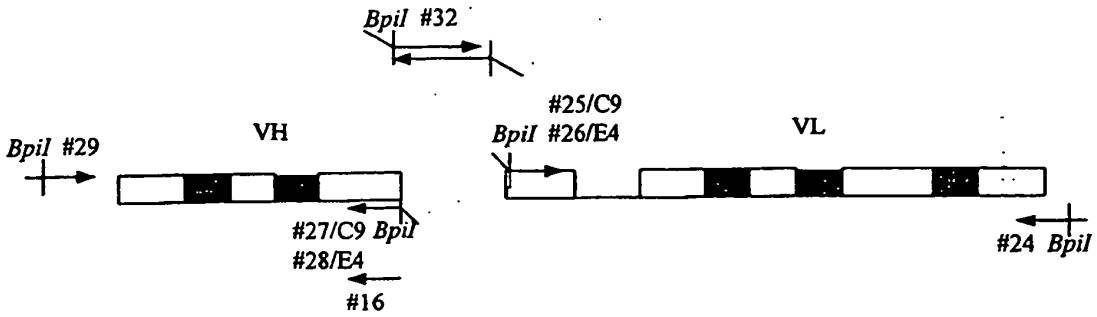
*Nco*I
1 ccatggcgca ggtgcagctg caggagtctg ggggaggctt ggtacagcct gggggtccc
61 tgagactctc ctgtgcagcc tctggattca tgtttagcag gtatgccatg agctgggtcc
121 gccaggctcc agggaagggg ccagagtggg tctcaggtat tagtggtagt ggtggtagta
181 catactacgc agactccgtg aagggccggt tcaccgtctc cagagacaat tccaagaaca
241 cgctgtatct gcaaatgaac agcctgagag ccgaggacac ggccgtatat tactgtgcga
301 aagatctggg ctactatggt tcggggagcc aacccttga gtactggggc cagggaactc
*Hind*III
361 tggtcaccgt ctcctcaggg agtgcatccg ccccaaagct tgaagaaggt gaattttcag
*Mlu*I
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 cagaagtggt aatcatgtaa
721 atgtgctatt cggcggaggg accaagctga ccgtcctacg tcagcccaag gctgcccccc
*Not*I
781 cggtcactct gttcccgccc tcttctgcgg ccgc

Figure 3: DNAsequence of C9 and E4 scFv masterframeworks
Restriction endonuclease recognition sequences flanking heavy and light chains (*Nco*I, *Hind*III and *Mlu*I, *Not*I 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
C9/VHCDR1.2/back/cut (#27): TAC TAC GAA GAC GTG TCC TCA GGT CTC AGG CTG GTC E4/VHCDR1.2/back/cut (#28): TAC TAC GAA GAC GTG TCC TCG GCT CTC AGG CTG TTC VH/for/cut (#29): AAT GCA GGT ATC ACG AGG CCC TTT CGT CTT C VL/back/cut (#24): CAG CTC TGA TAT CTT TGG ATC CGT TTA GGT CTT CTT CTG C9/VL/for/cut (#25): TAC TAC GAA GAC TGG TCA CCG TCT CCT CAG CCT CCA E4/VL/for/cut (#26): TAC TAC GAA GAC TGG TCA CCG TCT CCT CAG GGA GTG VHCDR3/stuff/for (#32): GGA CAC GTC TTC AGC GCT GAG CTC GAA GAC TG VHCDR3/stuff/back (#33): TGA CCA GTC TTC GAG CTC AGC GCT GAA GAC GT	VHCDR3_3.4/cut: GAG GAC ACG GCT GTA TAT TAC TGT GCG ARA (NNK)4 TTT GAS TAC TGG GGC CAG GGA ACC CTG GTC ACC VHCDR3_3.5/cut: GAG GAC ACG GCT GTA TAT TAC TGT GCG ARA (NNK)5 TTT GAS TAC TGG GGC CAG GGA ACC CTG GTC ACC VHCDR3_3.6/cut: GAG GAC ACG GCT GTA TAT TAC TGT GCG ARA (NNK)6 TTT GAS TAC TGG GGC CAG GGA ACC CTG GTC ACC VHCDR3_3.7/cut: GAG GAC ACG GCT GTA TAT TAC TGT GCG ARA (NNK)7 TTT GAS TAC TGG GGC CAG GGA ACC CTG GTC ACC VHCDR3_for/cut: AGC CTG GAA GAC GAG GAC ACG GCT GTA TAT TAC TGT GCG A VHCDR3_back/cut: GGC TGA GAA GAC GGT GAC CAG GGT TCC CTG GCC CCA GTA VHCDR3_ev1/for/cut (#42): AGC CTG GAA GAC GAG GAC ACG GCY GTG TAT TAC TGT VHCDR3_ev2/for/cut (#43): AGC CTG GAA GAC GAG GAC ACW GCC GTG TAT TAC TGT VHCDR3_ev3/for/cut (#44): AGC CTG GAA GAC GAG GAC ACG GCC GTA TAT TAC TGT VHCDR3_ev/back/cut (#45): GGC TGA GAA GAC GGT GAC CAG GGT KCC CTG GCC CCA

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). *Bpi*I restriction endonuclease recognition sites are indicated.

Fig. 5

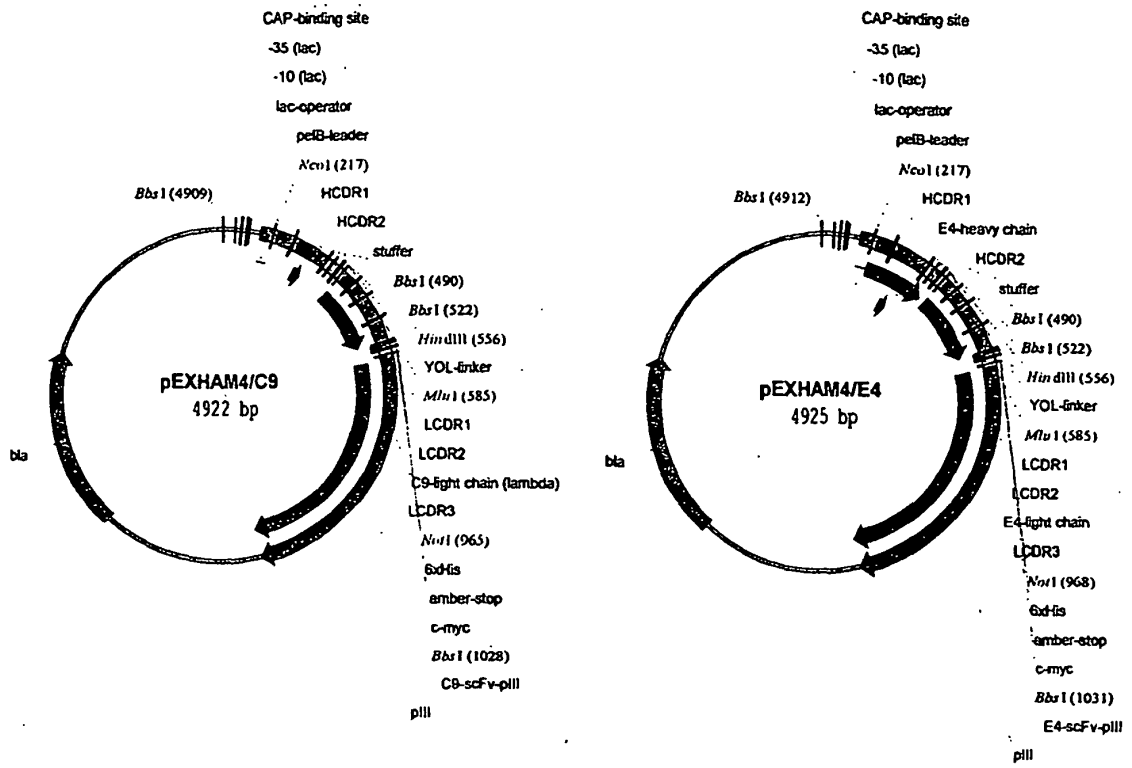


Figure 6: Vectormaps of pEXHAM4/C9 and pEXHAM4/E4

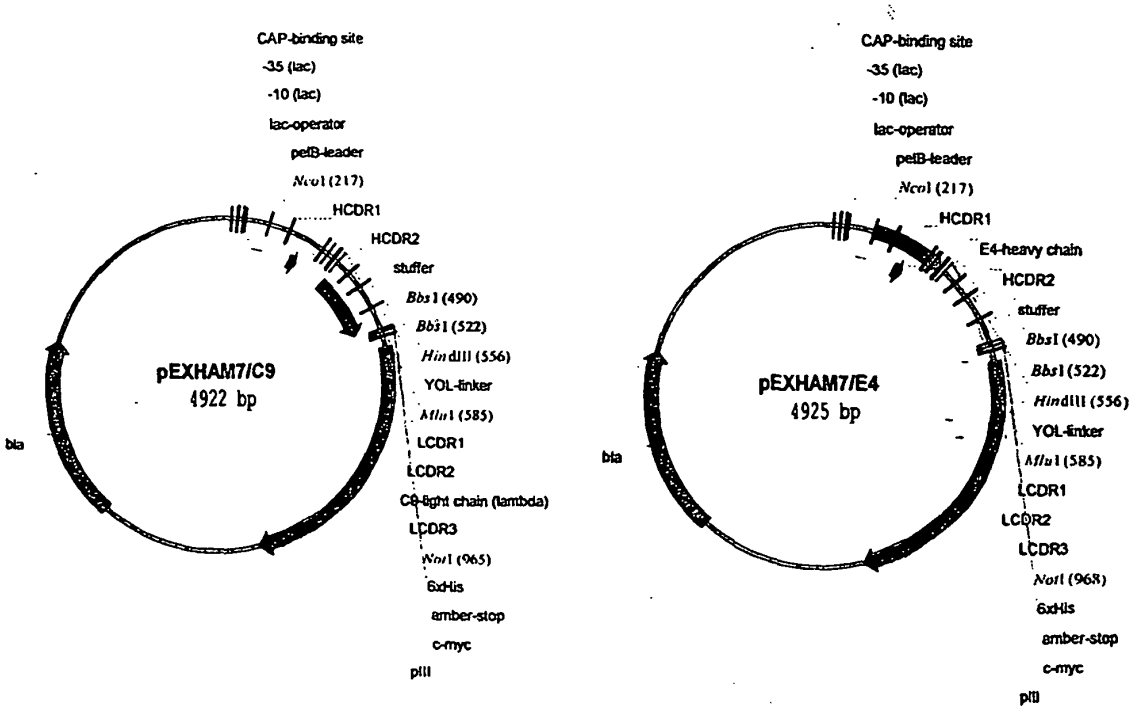


Figure 7: Vectormaps of pEXHAM7/C9 and pEXHAM7/E4

Oligonucleotides used as primers in 1. PCR for amplification of human heavy and light chain variable regions

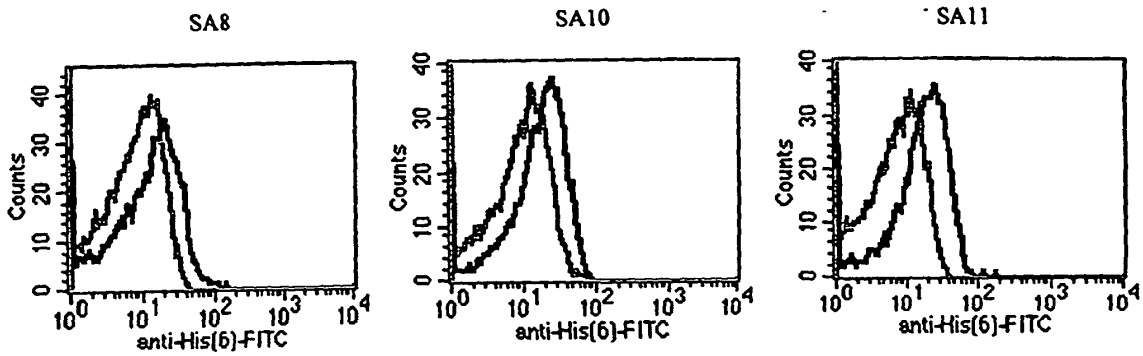
heavy chain primer			light chain primer		
VH-1a.	CAG GTG CAG CTG GTG CAG TCT	22	Vλ-1a.	CAG TCT GTG CTG ACG CAG CCA	39
VH-1b.	CAG GTC CAG CTT GTG CAG TCT		Vλ-1b.	CAG TCT GTG CTG ACG CAG CCG	
VH-1c.	CAG GTC CAG CTG GTA CAG TCT		Vλ-2	CAG TCT GCC CTG ACT CAG CCT	
VH-1d.	GAG GTC CAG CTG GTA CAG TCT		Vλ-3a.	TCC TAT GAG CTG ACA CAG CCA	
VH-1e.	CAG ATG CAG CTG GTA CAG TCT		Vλ-3b.	TCC TCT GAG CTG ACA CAG GAC	
VH-2a.	CAG ATC ACC TTG AAG GAG TCT		Vλ-3c.	TCC TAT GTG CTG ACA CAG CCA	
VH-2b.	CAG GTC ACC TTG AAG GAG TCT		Vλ-3d.	TCC TAT GAG CTG ACA CAG CTA	
VH-3a.	GAA GTG CAG CTG GTG GAG TCT		Vλ-3e.	TCC TAT GAG CTG ATG CAG CCA	
VH-3b.	CAG GTG CAG CTG GTG GAG TCT		Vλ-4a.	CTG CCT GTG CTG ACT CAG CCC	
VH-3c.	GAG GTG CAG CTG TTG GAG TCT		Vλ-4b.	CAG CCT GTG CTG ACT CAA TCA	
VH-4a.	CAG GTG CAG CTG CAG GAG TCG		Vλ-4c.	CAG CTT GTG CTG ACT CAA TCG	
VH-4b.	CAG CTG CAG CTG CAG GAG TCG		Vλ-5a.	CAG CCT GTG CTG ACT CAG CCA	
VH-4c.	CAG GTG CAG CTA CAG CAG TGG		Vλ-5b.	CAG GCT GTG CTG ACT CAG CCG	
VH-5	GAA GTG CAG CTG GTG CAG TCT		Vλ-6	AAT TTT ATG CTG ACT CAG CCC	
VH-6	CAG GTA CAG CTG CAG CAG TCA		Vλ-7a.	CAG ACT GTG GTG ACT CAG GAG	
VH-7	CAG GTG CAG CTG GTG CAA TCT		Vλ-7b.	CAG GCT GTG GTG ACT CAG GAG	
			Vλ-8	CAG ACT GTG GTG ACC CAG GAG	
IgM	AAG GGT TGG GGC GGA TGC ACT	38	Vλ-9	CAG CCT GTG CTG ACT CAG CCA	
			Vλ-10	CAG GCA GGG CTG ACT CAG CCA	
			Vκ-1a.	GAC ATC CAG ATG ACC CAG TCT	
			Vκ-1b.	AAC ATC CAG ATG ACC CAG TCT	
			Vκ-1c.	GCC ATC CAG TTG ACC CAG TCT	
			Vκ-1d.	GAC ATC CAG TTG ACC CAG TCT	
			Vκ-1e.	GCC ATC CGG ATG ACC CAG TCT	
			Vκ-1f.	GTC ATC TGG ATG ACC CAG TCT	
			Vκ-1g.	GCC ATC CAG ATG ACC CAG TCT	
			Vκ-2a.	GAT ATT GTG ATG ACC CAG ACT	
			Vκ-2b.	GAT GTT GTG ATG ACT CAG TCT	
			Vκ-2c.	GAT ATT GTG ATG ACT CAG TCT	
			Vκ-3a.	GAA ATT GTG TTG ACG CAG TCT	
			Vκ-3b.	GAA ATT GTG ATG ACG CAG TCT	
			Vκ-3c.	GAA ATT GTA ATG ACG CAG TCT	
			Vκ-4	GAC ATC GTG ATG ACC CAG TCT	
			Vκ-5	GAA ACG ACA CTC ACG CAG TCT	
			Vκ-6a.	GAA ATT GTG CTG ACT CAG TCT	
			Vκ-6b.	GAT GTT GTG ATG ACA CAG TCT	
			C-λ	GGA CGG.CGG.GAA.CAG.AGT.GAC	
			C-κ	GAC AGA TGG TGC AGC CAC AGT	37

Fig. 8

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

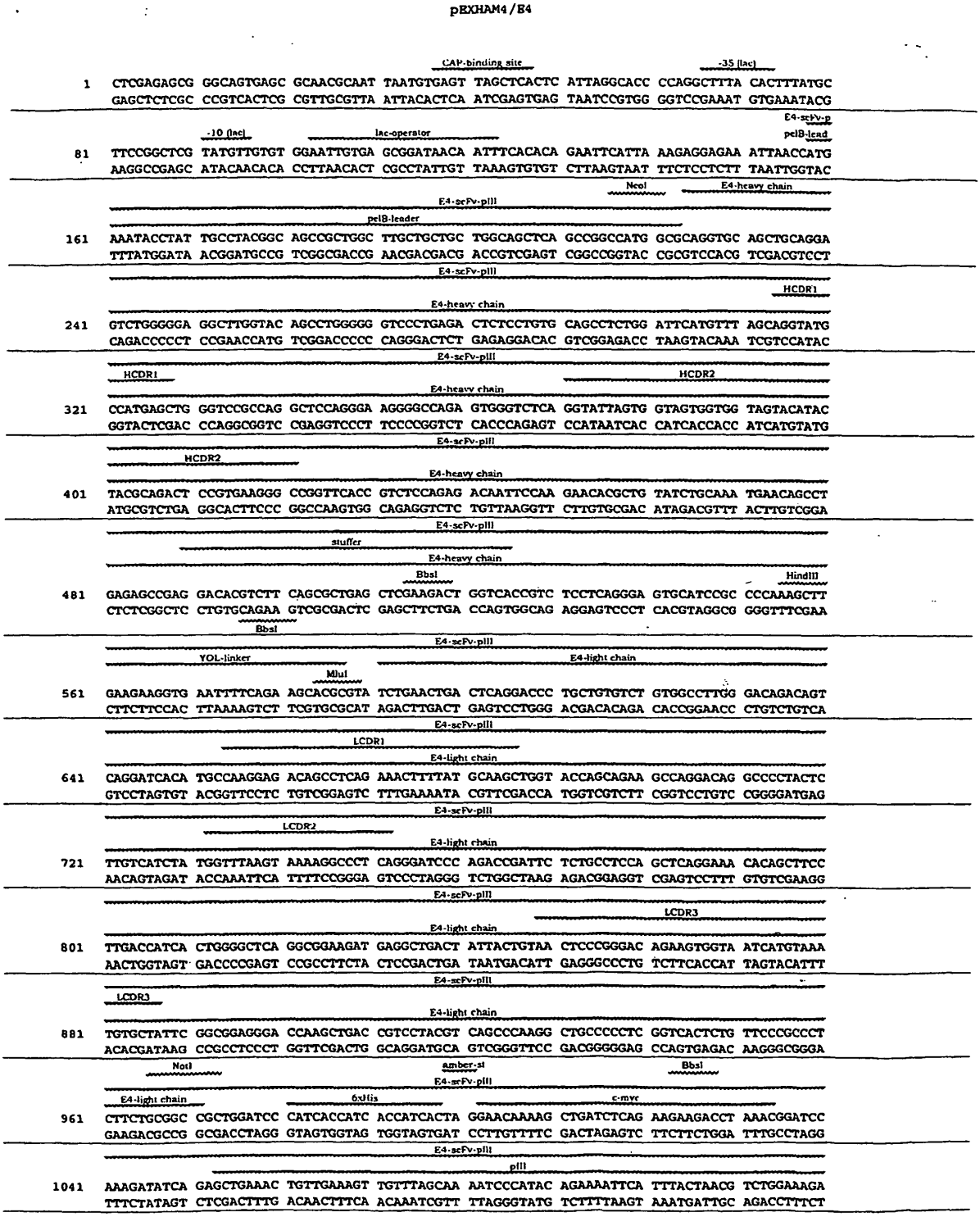
heavy chain primer		light chain primer	
	NcoI		MluI
VH-1a.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG CAG TCT	Vλ-1a.	CCT ACA GAA CGC GTA CAG TCT GTG CTG ACG CAG CCA
VH-1b.	TGG ACG CCC ATG GCG CAG GTC CAG CTT GTG CAG TCT	Vλ-1b.	CCT ACA GAA CGC GTA CAG TCT GTG CTG ACG CAG CCG
VH-1c.	TGG ACG CCC ATG GCG CAG GTC CAG CTG GTA CAG TCT	Vλ-2	CCT ACA GAA CGC GTA CAG TCT GCC CTG ACT CAG CCT
VH-1d.	TGG ACG CCC ATG GCG GAG GTC CAG CTG GTA CAG TCT	Vλ-3a.	CCT ACA GAA CGC GTA TCC TAT GAG CTG ACA CAG CCA
VH-1e.	TGG ACG CCC ATG GCG CAG ATG CAG CTG GTA CAG TCT	Vλ-3b.	CCT ACA GAA CGC GTA TCC TCT GAG CTG ACA CAG GAC
VH-2a.	TGG ACG CCC ATG GCG CAG ATC ACC TTG AAG GAG TCT	Vλ-3c.	CCT ACA GAA CGC GTA TCC TAT GTG CTG ACA CAG CCA
VH-2b.	TGG ACG CCC ATG GCG CAG GTC ACC TTG AAG GAG TCT	Vλ-3d.	CCT ACA GAA CGC GTA TCC TAT GAG CTG ACA CAG CTA
VH-3a.	TGG ACG CCC ATG GCG GAA GTG CAG CTG GTG GAG TCT	Vλ-3e.	CCT ACA GAA CGC GTA TCC TAT GAG CTG ATG CAG CCA
VH-3b.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG GAG TCT	Vλ-4a.	CCT ACA GAA CGC GTA CTG CCT GTG CTG ACT CAG CCC
VH-3c.	TGG ACG CCC ATG GCG GAG GTG CAG CTG TTG GAG TCT	Vλ-4b.	CCT ACA GAA CGC GTA CAG CCT GTG CTG ACT CAA TCA
VH-4a.	TGG ACG CCC ATG GCG CAG GTG CAG CTG CAG GAG TCG	Vλ-4c.	CCT ACA GAA CGC GTA CAG CTT GTG CTG ACT CAA TCG
VH-4b.	TGG ACG CCC ATG GCG CAG CTG CAG CTG CAG GAG TCG	Vλ-5a.	CCT ACA GAA CGC GTA CAG CCT GTG CTG ACT CAG CCA
VH-4c.	TGG ACG CCC ATG GCG CAG GTG CAG CTA CAG CAG TGG	Vλ-5b.	CCT ACA GAA CGC GTA CAG GCT GTG CTG ACT CAG CCG
VH-5	TGG ACG CCC ATG GCG GAA GTG CAG CTG GTG CAG TCT	Vλ-6	CCT ACA GAA CGC GTA AAT TTT ATG CTG ACT CAG CCC
VH-6	TGG ACG CCC ATG GCG CAG GTA CAG CTG CAG CAG TCA	Vλ-7a.	CCT ACA GAA CGC GTA CAG ACT GTG GTG ACT CAG GAG
VH-7	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG CAA TCT	Vλ-7b.	CCT ACA GAA CGC GTA CAG GCT GTG GTG ACT CAG GAG
	HindIII	Vλ-8	CCT ACA GAA CGC GTA CAG ACT GTG GTG ACC CAG GAG
IgM	TGG GAA AAG CTT AAG GGT TGG GGC GGA TGC ACT	Vλ-9	CCT ACA GAA CGC GTA CAG CCT GTG CTG ACT CAG CCA
		Vλ-10	CCT ACA GAA CGC GTA CAG GCA GGG CTG ACT CAG CCA
		Vκ-1a.	CCT ACA GAA CGC GTA GAC ATC CAG ATG ACC CAG TCT
		Vκ-1b.	CCT ACA GAA CGC GTA AAC ATC CAG ATG ACC CAG TCT
		Vκ-1c.	CCT ACA GAA CGC GTA GCC ATC CAG TTG ACC CAG TCT
		Vκ-1d.	CCT ACA GAA CGC GTA GAC ATC CAG TTG ACC CAG TCT
		Vκ-1e.	CCT ACA GAA CGC GTA GCC ATC CGG ATG ACC CAG TCT
		Vκ-1f.	CCT ACA GAA CGC GTA GTC ATC TGG ATG ACC CAG TCT
		Vκ-1g.	CCT ACA GAA CGC GTA GCC ATC CAG ATG ACC CAG TCT
		Vκ-2a.	CCT ACA GAA CGC GTA GAT ATT GTG ATG ACC CAG ACT
		Vκ-2b.	CCT ACA GAA CGC GTA GAT GTT GTG ATG ACT CAG TCT
		Vκ-2c.	CCT ACA GAA CGC GTA GAT ATT GTG ATG ACT CAG TCT
		Vκ-3a.	CCT ACA GAA CGC GTA GAA ATT GTG TTG ACG CAG TCT
		Vκ-3b.	CCT ACA GAA CGC GTA GAA ATT GTG ATG ACG CAG TCT
		Vκ-3c.	CCT ACA GAA CGC GTA GAA ATT GTA ATG ACG CAG TCT
		Vκ-4	CCT ACA GAA CGC GTA GAC ATC GTG ATG ACC CAG TCT
		Vκ-5	CCT ACA GAA CGC GTA GAA ACG ACA CTC ACG CAG TCT
		Vκ-6a.	CCT ACA GAA CGC GTA GAA ATT GTG CTG ACT CAG TCT
		Vκ-6b.	CCT ACA GAA CGC GTA GAT GTT GTG ATG ACA CAG TCT
			NotI
		C-λ	GGG CGG CAG GGC GGC CGC GGA CGG CGG GAA CAG AGT GAC
		C-κ	GGG CGG CAG GGC GGC CGC GAC AGA TGG TGC AGC CAC AGT

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


pEXHAM4/E4
CAP-binding site
-35 (lac)
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GAGCTCTCGC CCGTCACTCG CGTTGCGTTA ATTACACTCA ATCGAGTGAG TAATCCGTGG GGTCCGAAAT GTGAAATACG
E4-scFv-p
-10 (lac)
lac-operator
pelB-lead
81 TTCCGGCTCG TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTCACACA GAATTCATTA AAGAGGAGAA ATTAACCATG
AAGGCCGAGC ATACAACACA CCTTAACACT CGCCTATTGT TAAAGTGTGT CTTAAGTAAT TTCTCCTCTT TAATTGGTAC
NcoI
E4-heavy chain
E4-scFv-pIII
pelB-leader
161 AAATACCTAT TGCCTACGGC AGCCGCTGGC TTGCTGCTGC TGGCAGCTCA GCCGGCCATG GCGCAGGTGC AGCTGCAGGA
TTTATGGATA ACGGATGCCG TCGGCGACCG AACGACGACG ACCGTCGAGT CGGCCGGTAC CGCGTCCACG TCGACGTCCT
E4-scFv-pIII
HCDR1
E4-heavy chain
241 GTCTGGGGGA GGCTTGGTAC AGCCTGGGGG GTCCCTGAGA CTCTCCTGTG CAGCCTCTGG ATTCATGTTT AGCAGGTATG
CAGACCCCCT CCGAACCATG TCGGACCCCC CAGGGACTCT GAGAGGACAC GTCGGAGACC TAAGTACAAA TCGTCCATAC
E4-scFv-pIII
HCDR1
HCDR2
E4-heavy chain
321 CCATGAGCTG GGTCCGCCAG GCTCCAGGGA AGGGGCCAGA GTGGGTCTCA GGTATTAGTG GTAGTGGTGG TAGTACATAC
GGTACTCGAC CCAGGCGGTC CGAGGTCCCT TCCCCGGTCT CACCCAGAGT CCATAATCAC CATCACCACC ATCATGTATG
E4-scFv-pIII
HCDR2
E4-heavy chain
401 TACGCAGACT CCGTGAAGGG CCGGTTCACC GTCTCCAGAG ACAATTCCAA GAACACGCTG TATCTGCAAA TGAACAGCCT
ATGCGTCTGA GGCACTTCCC GGCCAAGTGG CAGAGGTCTC TGTTAAGGTT CTTGTGCGAC ATAGACGTTT ACTTGTCGGA
E4-scFv-pIII
stuffer
E4-heavy chain
BbsI
HindIII
481 GAGAGCCGAG GACACGTCTT CAGCGCTGAG CTCGAAGACT GGTCACCGTC TCCTCAGGGA GTGCATCCGC CCCAAAGCTT
CTCTCGGCTC CTGTGCAGAA GTCGCGACTC GAGCTTCTGA CCAGTGGCAG AGGAGTCCCT CACGTAGGCG GGGTTTCGAA
BbsI
E4-scFv-pIII
VOL-linker
E4-light chain
MluI
561 GAAGAAGGTG AATTTTCAGA AGCACGCGTA TCTGAACTGA CTCAGGACCC TGCTGTGTCT GTGGCCTTGG GACAGACAGT
CTTCTTCCAC TTAAAAGTCT TCGTGCGCAT AGACTTGACT GAGTCCTGGG ACGACACAGA CACCGGAACC CTGTCTGTCA
E4-scFv-pIII
LCDR1
E4-light chain
641 CAGGATCACA TGCCAAGGAG ACAGCCTCAG AAACTTTTAT GCAAGCTGGT ACCAGCAGAA GCCAGGACAG GCCCCTACTC
GTCCTAGTGT ACGGTTCCTC TGTCGGAGTC TTTGAAAATA CGTTCGACCA TGGTCGTCTT CGGTCCTGTC CGGGGATGAG
E4-scFv-pIII
LCDR2
E4-light chain
721 TTGTCATCTA TGGTTTAAGT AAAAGGCCCT CAGGGATCCC AGACCGATTC TCTGCCTCCA GCTCAGGAAA CACAGCTTCC
AACAGTAGAT ACCAAATTCA TTTTCCGGGA GTCCCTAGGG TCTGGCTAAG AGACGGAGGT CGAGTCCTTT GTGTCGAAGG
E4-scFv-pIII
LCDR3
E4-light chain
801 TTGACCATCA CTGGGGCTCA GGCGGAAGAT GAGGCTGACT ATTACTGTAA CTCCCGGGAC AGAAGTGGTA ATCATGTAAA
AACTGGTAGT GACCCCGAGT CCGCCTTCTA CTCCGACTGA TAATGACATT GAGGGCCCTG TCTTCACCAT TAGTACATTT
E4-scFv-pIII
LCDR3
E4-light chain
881 TGTGCTATTC GGCGGAGGGA CCAAGCTGAC CGTCCTACGT CAGCCCAAGG CTGCCCCCTC GGTCACTCTG TTCCCGCCCT
ACACGATAAG CCGCCTCCCT GGTTCGACTG GCAGGATGCA GTCGGGTTCC GACGGGGGAG CCAGTGAGAC AAGGGCGGGA
NotI
amber-sI
BbsI
E4-scFv-pIII
E4-light chain
6xHis
c-myc
961 CTTCTGCGGC CGCTGGATCC CATCACCATC ACCATCACTA GGAACAAAAG CTGATCTCAG AAGAAGACCT AAACGGATCC
GAAGACGCCG GCGACCTAGG GTAGTGGTAG TGGTAGTGAT CCTTGTTTTC GACTAGAGTC TTCTTCTGGA TTTGCCTAGG
E4-scFv-pIII
pIII
1041 AAAGATATCA GAGCTGAAAC TGTTGAAAGT TGTTTAGCAA AATCCCATAC AGAAAATTCA TTTACTAACG TCTGGAAAGA
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Fig. 11a

pEXHAM4/E4

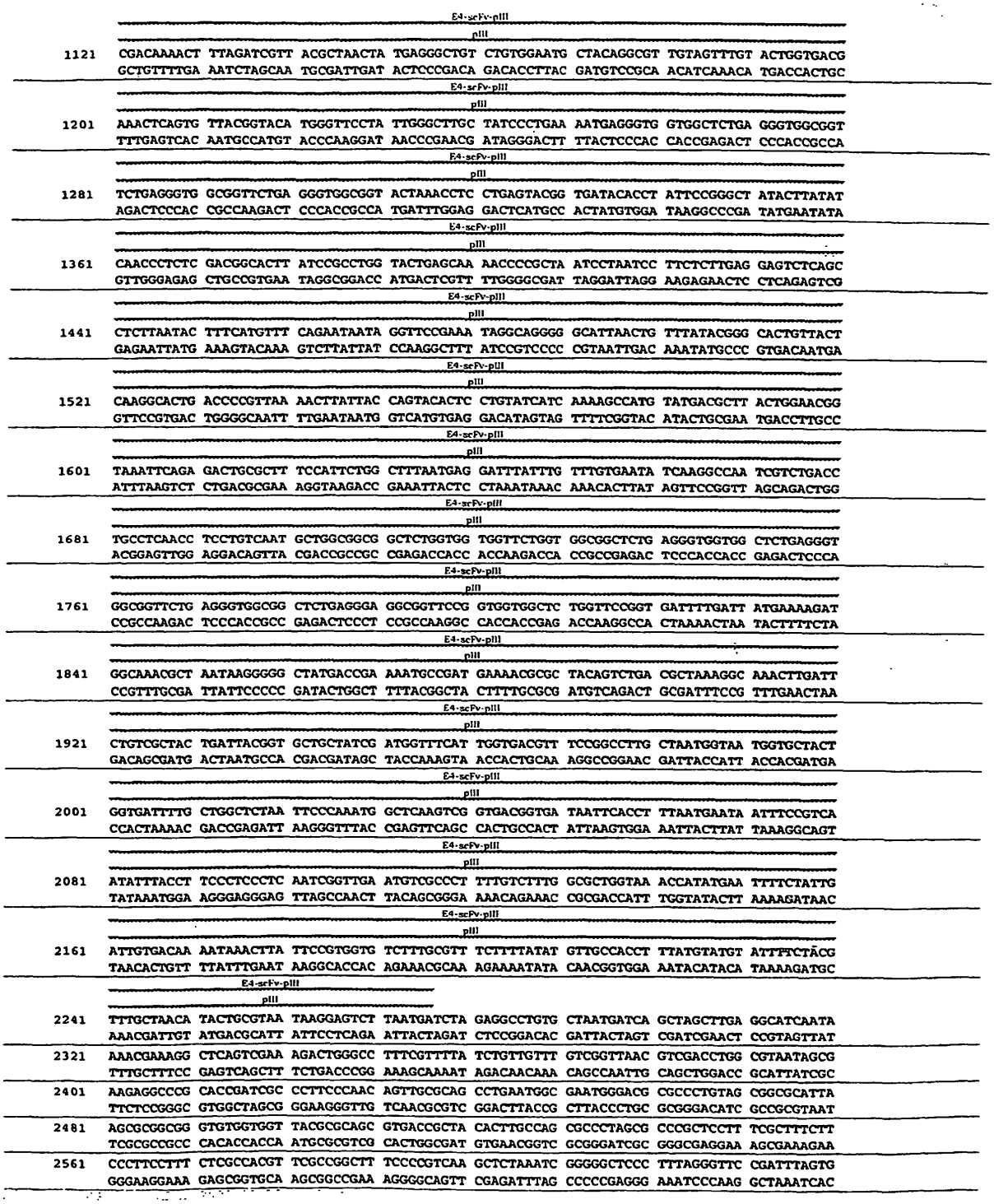

Fig. 11b

pEXHAM4/E4

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2721  CGCCCTTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGT
      GCGGGAAACT GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCA
2801  CTATTCTTTT GATTTATAAG GGATTTTGCC GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG
      GATAAGAAAA CTAAATATTC CCTAAAACGG CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC
2881  CGAATTTTAA CAAAATATTA ACGCTTACAA TTTAGGTGGC ACTTTTCGGG GAAATGTGCG CGGAACCCCT ATTTGTTTAT
      GCTTAAAATT GTTTTATAAT TGCGAATGTT AAATCCACCG TGAAAAGCCC CTTTACACGC GCCTTGGGGA TAAACAAATA
2961  TTTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA ATAACCCTGA TAAATGCTTC AATAATATTG AAAAAGGAAG
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                                                                                    bla
3041  AGTATGAGTA TTCAACATTT CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA
      TCATACTCAT AAGTTGTAAA GGCACAGCGG GAATAAGGGA AAAAACGCCG TAAAACGGAA GGACAAAAAC GAGTGGGTCT
                                                                                    bla
3121  AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA
      TTGCGACCAC TTTCATTTTC TACGACTTCT AGTCAACCCA CGTGCTCACC CAATGTAGCT TGACCTAGAG TTGTCGCCAT
                                                                                    bla
3201  AGATCCTTGA GAGTTTTCGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC TGCTATGTGG CGCGGTATTA
      TCTAGGAACT CTCAAAAGCG GGGCTTCTTG CAAAAGGTTA CTACTCGTGA AAATTTCAAG ACGATACACC GCGCCATAAT
                                                                                    bla
3281  TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
      AGGGCATAAC TGCGGCCCGT TCTCGTTGAG CCAGCGGCGT ATGTGATAAG AGTCTTACTG AACCAACTCA TGAGTGGTCA
                                                                                    bla
3361  CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG
      GTGTCTTTTC GTAGAATGCC TACCGTACTG TCATTCTCTT AATACGTCAC GACGGTATTG GTACTCACTA TTGTGACGCC
                                                                                    bla
3441  CCAACTTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC
      GGTTGAATGA AGACTGTTGC TAGCCTCCTG GCTTCCTCGA TTGGCGAAAA AACGTGTTGT ACCCCCTAGT ACATTGAGCG
                                                                                    bla
3521  CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC
      GAACTAGCAA CCCTTGGCCT CGACTTACTT CGGTATGGTT TGCTGCTCGC ACTGTGGTGC TACGGACATC GTTACCGTTG
                                                                                    bla
3601  AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT AGACTGGATG GAGGCGGATA
      TTGCAACGCG TTTGATAATT GACCGCTTGA TGAATGAGAT CGAAGGGCCG TTGTTAATTA TCTGACCTAC CTCCGCCTAT
                                                                                    bla
3681  AAGTTGCAGG ACCACTTCTG CGCTCGGCCC TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
      TTCAACGTCC TGGTGAAGAC GCGAGCCGGG AAGGCCGACC GACCAAATAA CGACTATTTA GACCTCGGCC ACTCGCACCC
                                                                                    bla
3761  TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG GGAGTCAGGC
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                                                                                    bla
3841  AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAACTGTCA GACCAAGTTT
      TTGATACCTA CTTGCTTTAT CTGTCTAGCG ACTCTATCCA CGGAGTGACT AATTCGTAAC CATTGACAGT CTGGTTCAAA
3921  ACTCATATAT ACTTTAGATT GATTTAAAAC TTCATTTTTA ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC
      TGAGTATATA TGAAATCTAA CTAAATTTTG AAGTAAAAAT TAAATTTTCC TAGATCCACT TCTAGGAAAA ACTATTAGAG
4001  ATGACCAAAA TCCCTTAACG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA
      TACTGGTTTT AGGGAATTGC ACTCAAAAGC AAGGTGACTC GCAGTCTGGG GCATCTTTTC TAGTTTCCTA GAAGAACTCT
4081  TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC TACCAGCGGT GGTTTGTTTG CCGGATCAAG
      AGGAAAAAAA GACGCGCATT AGACGACGAA CGTTTGTTTT TTTGGTGGCG ATGGTCGCCA CCAAACAAAC GGCCTAGTTC
4161  AGCTACCAAC TCTTTTTCCG AAGGTAACTG GCTTCAGCAG AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG
      TCGATGGTTG AGAAAAAGGC TTCCATTGAC CGAAGTCGTC TCGCGTCTAT GGTTTATGAC AGGAAGATCA CATCGGCATC
4241  TTAGGCCACC ACTTCAAGAA CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG
      AATCCGGTGG TGAAGTTCTT GAGACATCGT GGCGGATGTA TGGAGCGAGA CGATTAGGAC AATGGTCACC GACGACGGTC
4321  TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA GCGGTCGGGC TGAACGGGGG
      ACCGCTATTC AGCACAGAAT GGCCCAACCT GAGTTCTGCT ATCAATGGCC TATTCCGCGT CGCCAGCCCG ACTTGCCCCC
4401  GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA CGACCTACAC CGAACTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC
      CAAGCACGTG TGTCGGGTCG AACCTCGCTT GCTGGATGTG GCTTGACTCT ATGGATGTCG CACTCGATAC TCTTTCGCGG
4481  ACGCTTCCCG AAGGGAGAAA GGCGGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC
      TGCGAAGGGC TTCCCTCTTT CCGCCTGTCC ATAGGCCATT CGCCGTCCCA GCCTTGTCCT CTCGCGTGCT CCCTCGAAGG
4561  AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GACTTGAGCG TCGATTTTTG TGATGCTCGT
      TCCCCCTTTG CGGACCATAG AAATATCAGG ACAGCCCAAA GCGGTGGAGA CTGAACTCGC AGCTAAAAAC ACTACGAGCA
4641  CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC
      GTCCCCCCGC CTCGGATACC TTTTTGCGGT CGTTGCGCCG GAAAAATGCC AAGGACCGGA AAACGACCGG AAAACGAGTG
4721  ATGTTCTTTC CTGCGTTATC CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGCAG
      TACAAGAAAG GACGCAATAG GGGACTAAGA CACCTATTGG CATAATGGCG GAAACTCACT CGACTATGGC GAGCGGCGTC
```

Fig. 11c

pEXHAM4/B4

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4801  CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC CAATACGCAA ACCGCCTCTC CCCGCGCGTT
      GGCTTGCTGG CTCGCGTCGC TCAGTCACTC GCTCCTTCGC CTTCTCGCGG GTTATGCGTT TGGCGGAGAG GGGCGCGCAA

4881  GGCCGATTCA TTAATGCAGG TATCACGAGG CCCTTTCGTC TTCAC
      CCGGCTAAGT AATTACGTCC ATAGTGCTCC GGGAAAGCAG AAGTG
                                 BbsI
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Fig. 11d

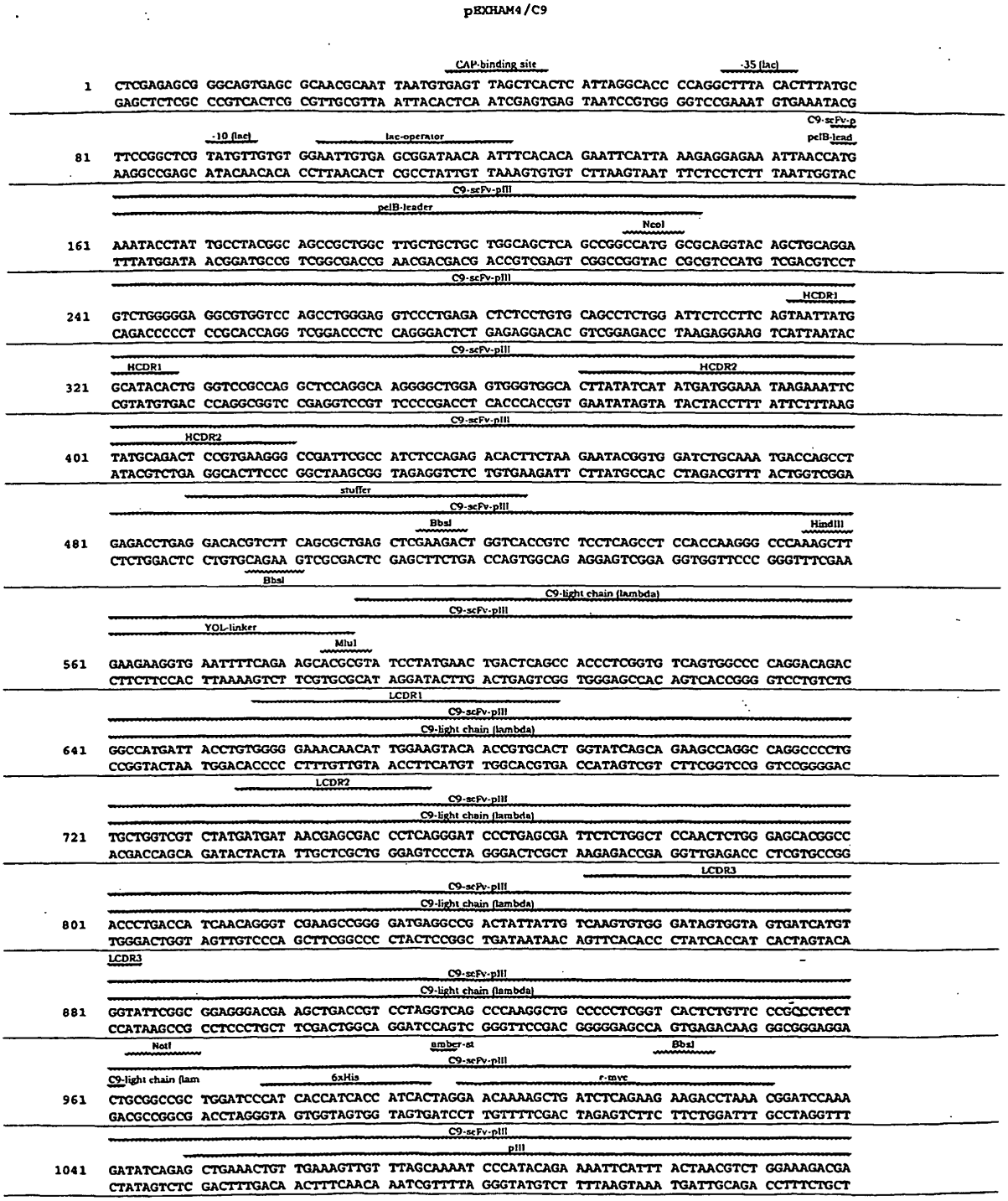
pEXHAM4/C9
CAP-binding site
-35 (lac)
1 CTCGAGAGCG GGCAGTGAGC GCAACGCAAT TAATGTGAGT TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC
GAGCTCTCGC CCGTCACTCG CGTTGCGTTA ATTACACTCA ATCGAGTGAG TAATCCGTGG GGTCCGAAAT GTGAAATACG
C9-scFv-p
-10 (lac)
lac-operator
pelB-lead
81 TTCCGGCTCG TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTCACACA GAATTCATTA AAGAGGAGAA ATTAACCATG
AAGGCCGAGC ATACAACACA CCTTAACACT CGCCTATTGT TAAAGTGTGT CTTAAGTAAT TTCTCCTCTT TAATTGGTAC
C9-scFv-pIII
pelB-leader
NcoI
161 AAATACCTAT TGCCTACGGC AGCCGCTGGC TTGCTGCTGC TGGCAGCTCA GCCGGCCATG GCGCAGGTAC AGCTGCAGGA
TTTATGGATA ACGGATGCCG TCGGCGACCG AACGACGACG ACCGTCGAGT CGGCCGGTAC CGCGTCCATG TCGACGTCCT
C9-scFv-pIII
HCDR1
241 GTCTGGGGGA GGCGTGGTCC AGCCTGGGAG GTCCCTGAGA CTCTCCTGTG CAGCCTCTGG ATTCTCCTTC AGTAATTATG
CAGACCCCCT CCGCACCAGG TCGGACCCTC CAGGGACTCT GAGAGGACAC GTCGGAGACC TAAGAGGAAG TCATTAATAC
C9-scFv-pIII
HCDR1
HCDR2
321 GCATACACTG GGTCCGCCAG GCTCCAGGCA AGGGGCTGGA GTGGGTGGCA CTTATATCAT ATGATGGAAA TAAGAAATTC
CGTATGTGAC CCAGGCGGTC CGAGGTCCGT TCCCCGACCT CACCCACCGT GAATATAGTA TACTACCTTT ATTCTTTAAG
C9-scFv-pIII
HCDR2
401 TATGCAGACT CCGTGAAGGG CCGATTCGCC ATCTCCAGAG ACACTTCTAA GAATACGGTG GATCTGCAAA TGACCAGCCT
ATACGTCTGA GGCACTTCCC GGCTAAGCGG TAGAGGTCTC TGTGAAGATT CTTATGCCAC CTAGACGTTT ACTGGTCGGA
stuffer
C9-scFv-pIII
BbsI
HindIII
481 GAGACCTGAG GACACGTCTT CAGCGCTGAG CTCGAAGACT GGTCACCGTC TCCTCAGCCT CCACCAAGGG CCCAAAGCTT
CTCTGGACTC CTGTGCAGAA GTCGCGACTC GAGCTTCTGA CCAGTGGCAG AGGAGTCGGA GGTGGTTCCC GGGTTTCGAA
BbsI
C9-light chain (lambda)
C9-scFv-pIII
YOL-linker
MluI
561 GAAGAAGGTG AATTTTCAGA AGCACGCGTA TCCTATGAAC TGACTCAGCC ACCCTCGGTG TCAGTGGCCC CAGGACAGAC
CTTCTTCCAC TTAAAAGTCT TCGTGCGCAT AGGATACTTG ACTGAGTCGG TGGGAGCCAC AGTCACCGGG GTCCTGTCTG
LCDR1
C9-scFv-pIII
C9-light chain (lambda)
641 GGCCATGATT ACCTGTGGGG GAAACAACAT TGGAAGTACA ACCGTGCACT GGTATCAGCA GAAGCCAGGC CAGGCCCCTG
CCGGTACTAA TGGACACCCC CTTTGTTGTA ACCTTCATGT TGGCACGTGA CCATAGTCGT CTTCGGTCCG GTCCGGGGAC
LCDR2
C9-scFv-pIII
C9-light chain (lambda)
721 TGCTGGTCGT CTATGATGAT AACGAGCGAC CCTCAGGGAT CCCTGAGCGA TTCTCTGGCT CCAACTCTGG GAGCACGGCC
ACGACCAGCA GATACTACTA TTGCTCGCTG GGAGTCCCTA GGGACTCGCT AAGAGACCGA GGTTGAGACC CTCGTGCCGG
LCDR3
C9-scFv-pIII
C9-light chain (lambda)
801 ACCCTGACCA TCAACAGGGT CGAAGCCGGG GATGAGGCCG ACTATTATTG TCAAGTGTGG GATAGTGGTA GTGATCATGT
TGGGACTGGT AGTTGTCCCA GCTTCGGCCC CTACTCCGGC TGATAATAAC AGTTCACACC CTATCACCAT CACTAGTACA
LCDR3
C9-scFv-pIII
C9-light chain (lambda)
881 GGTATTCGGC GGAGGGACGA AGCTGACCGT CCTAGGTCAG CCCAAGGCTG CCCCCTCGGT CACTCTGTTC CCGCCCTCCT
CCATAAGCCG CCTCCCTGCT TCGACTGGCA GGATCCAGTC GGGTTCCGAC GGGGGAGCCA GTGAGACAAG GGCGGGAGGA
NotI
amber-st
BbsI
C9-scFv-pIII
C9-light chain (lam
6xHis
c-myc
961 CTGCGGCCGC TGGATCCCAT CACCATCACC ATCACTAGGA ACAAAAGCTG ATCTCAGAAG AAGACCTAAA CGGATCCAAA
GACGCCGGCG ACCTAGGGTA GTGGTAGTGG TAGTGATCCT TGTTTTCGAC TAGAGTCTTC TTCTGGATTT GCCTAGGTTT
C9-scFv-pIII
pIII
1041 GATATCAGAG CTGAAACTGT TGAAAGTTGT TTAGCAAAAT CCCATACAGA AAATTCATTT ACTAACGTCT GGAAAGACGA
CTATAGTCTC GACTTTGACA ACTTTCAACA AATCGTTTTA GGGTATGTCT TTTAAGTAAA TGATTGCAGA CCTTTCTGCT

Fig. 12a

pEXHAM4/C9

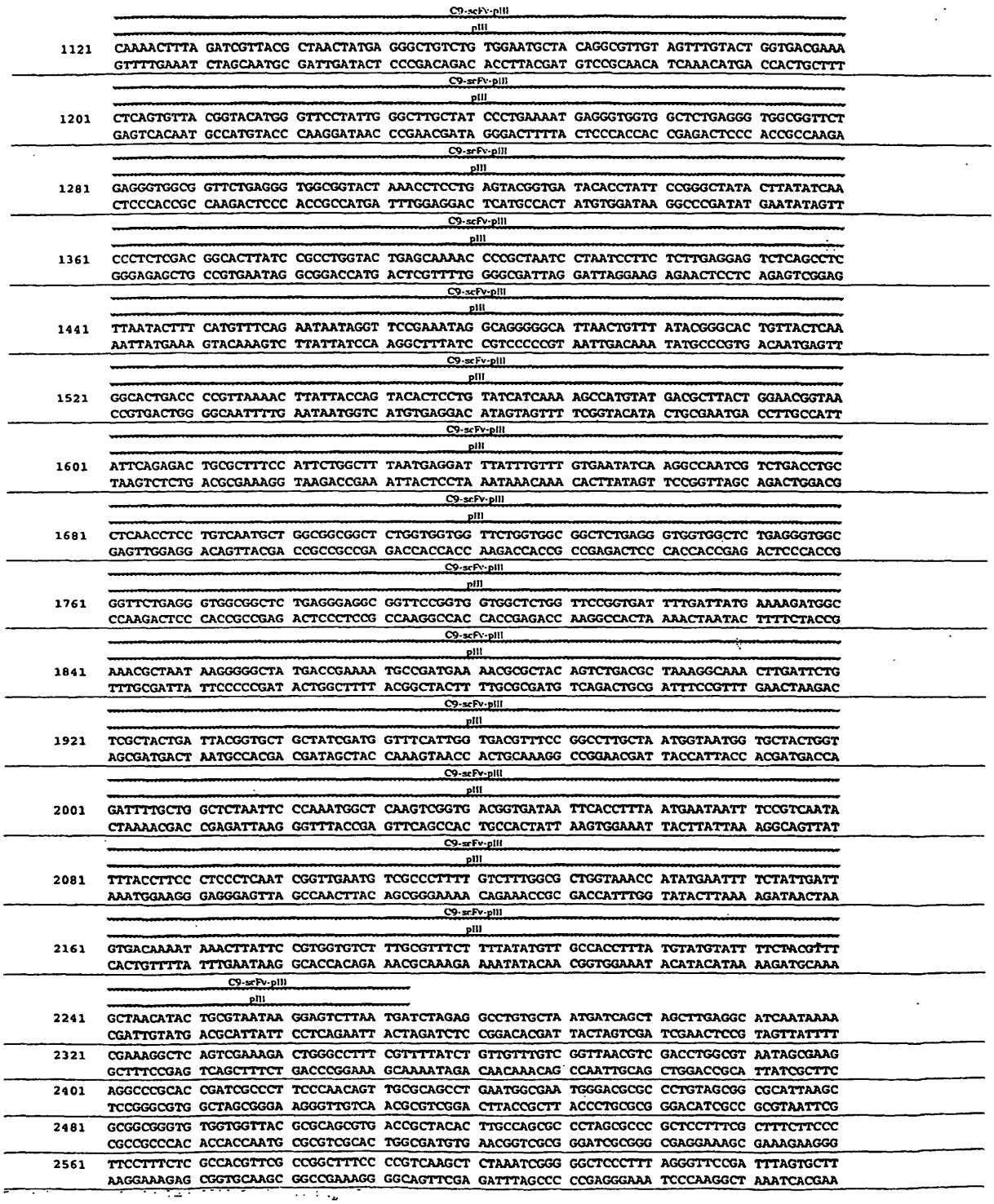

Fig. 12b

pEXHAM4/C9

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2641  TACGGCACCT CGACCCCAAA AAACTTGATT AGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC GGTTTTTCGC
      ATGCCGTGGA GCTGGGGTTT TTTGAACTAA TCCCACTACC AAGTGCATCA CCCGGTAGCG GGACTATCTG CCAAAAAGCG
2721  CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC TGGAACAACA CTCAACCCTA TCTCGGTCTA
      GGAAACTGCA ACCTCAGGTG CAAGAAATTA TCACCTGAGA ACAAGGTTTG ACCTTGTTGT GAGTTGGGAT AGAGCCAGAT
2801  TTCTTTTGAT TTATAAGGGA TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA TTTAACGCGA
      AAGAAAACTA AATATTCCCT AAAACGGCTA AAGCCGGATA ACCAATTTTT TACTCGACTA AATTGTTTTT AAATTGCGCT
2881  ATTTTAACAA AATATTAACG CTTACAATTT AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT
      TAAAATTGTT TTATAATTGC GAATGTTAAA TCCACCGTGA AAAGCCCCTT TACACGCGCC TTGGGGATAA ACAAATAAAA
2961  TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT
      AGATTTATGT AAGTTTATAC ATAGGCGAGT ACTCTGTTAT TGGGACTATT TACGAAGTTA TTATAACTTT TTCCTTCTCA
                                          bla
3041  ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC
      TACTCATAAG TTGTAAAGGC ACAGCGGGAA TAAGGGAAAA AACGCCGTAA AACGGAAGGA CAAAAACGAG TGGGTCTTTG
                                          bla
3121  GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA
      CGACCACTTT CATTTTCTAC GACTTCTAGT CAACCCACGT GCTCACCCAA TGTAGCTTGA CCTAGAGTTG TCGCCATTCT
                                          bla
3201  TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC
      AGGAACTCTC AAAAGCGGGG CTTCTTGCAA AAGGTTACTA CTCGTGAAAA TTTCAAGACG ATACACCGCG CCATAATAGG
                                          bla
3281  CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC
      GCATAACTGC GGCCCGTTCT CGTTGAGCCA GCGGCGTATG TGATAAGAGT CTTACTGAAC CAACTCATGA GTGGTCAGTG
                                          bla
3361  AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA
      TCTTTTCGTA GAATGCCTAC CGTACTGTCA TTCTCTTAAT ACGTCACGAC GGTATTGGTA CTCACTATTG TGACGCCGGT
                                          bla
3441  ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT
      TGAATGAAGA CTGTTGCTAG CCTCCTGGCT TCCTCGATTG GCGAAAAAAC GTGTTGTACC CCCTAGTACA TTGAGCGGAA
                                          bla
3521  GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGTAGCAA TGGCAACAAC
      CTAGCAACCC TTGGCCTCGA CTTACTTCGG TATGGTTTGC TGCTCGCACT GTGGTGCTAC GGACATCGTT ACCGTTGTTG
                                          bla
3601  GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG
      CAACGCGTTT GATAATTGAC CGCTTGATGA ATGAGATCGA AGGGCCGTTG TTAATTATCT GACCTACCTC CGCCTATTTC
                                          bla
3681  TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT
      AACGTCCTGG TGAAGACGCG AGCCGGGAAG GCCGACCGAC CAAATAACGA CTATTTAGAC CTCGGCCACT CGCACCCAGA
                                          bla
3761  CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC
      GCGCCATAGT AACGTCGTGA CCCCGGTCTA CCATTCGGGA GGGCATAGCA TCAATAGATG TGCTGCCCCT CAGTCCGTTG
                                          bla
3841  TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT
      ATACCTACTT GCTTTATCTG TCTAGCGACT CTATCCACGG AGTGACTAAT TCGTAACCAT TGACAGTCTG GTTCAAATGA
3921  CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG
      GTATATATGA AATCTAACTA AATTTTGAAG TAAAAATTAA ATTTTCCTAG ATCCACTTCT AGGAAAAACT ATTAGAGTAC
4001  ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC
      TGGTTTTAGG GAATTGCACT CAAAAGCAAG GTGACTCGCA GTCTGGGGCA TCTTTTCTAG TTTCCTAGAA GAACTCTAGG
4081  TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC
      AAAAAAAGAC GCGCATTAGA CGACGAACGT TTGTTTTTTT GGTGGCGATG GTCGCCACCA AACAAACGGC CTAGTTCTCG
4161  TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA
      ATGGTTGAGA AAAAGGCTTC CATTGACCGA AGTCGTCTCG CGTCTATGGT TTATGACAGG AAGATCACAT CGGCATCAAT
4241  GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG
      CCGGTGGTGA AGTTCTTGAG ACATCGTGGC GGATGTATGG AGCGAGACGA TTAGGACAAT GGTCACCGAC GACGGTCACC
4321  CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT
      GCTATTCAGC ACAGAATGGC CCAACCTGAG TTCTGCTATC AATGGCCTAT TCCGCGTCGC CAGCCCGACT TGCCCCCCAA
4401  CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG
      GCACGTGTGT CGGGTCGAAC CTCGCTTGCT GGATGTGGCT TGACTCTATG GATGTCGCAC TCGATACTCT TTCGCGGTGC
4481  CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG
      GAAGGGCTTC CCTCTTTCCG CCTGTCCATA GGCCATTCGC CGTCCCAGCC TTGTCCTCTC GCGTGCTCCC TCGAAGGTCC
4561  GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG
      CCCTTTGCGG ACCATAGAAA TATCAGGACA GCCCAAAGCG GTGGAGACTG AACTCGCAGC TAAAAACACT ACGAGCAGTC
4641  GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG
      CCCCCGCCTC GGATACCTTT TTGCGGTCGT TGCGCCGGAA AAATGCCAAG GACCGGAAAA CGACCGGAAA ACGAGTGTAC
4721  TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG
      AAGAAAGGAC GCAATAGGGG ACTAAGACAC CTATTGGCAT AATGGCGGAA ACTCACTCGA CTATGGCGAG CGGCGTCGGC
```

Fig. 12c

pEXHAM4/C9

```
4801   AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC
       TTGCTGGCTC GCGTCGCTCA GTCACTCGCT CCTTCGCCTT CTCGCGGGTT ATGCGTTTGG CGGAGAGGGG CGCGCAACCG
4881   CGATTCATTA ATGCAGGTAT CACGAGGCCC TTTCGTCTTC AC
       GCTAAGTAAT TACGTCCATA GTGCTCCGGG AAAGCAGAAG TG
                                        BbsI
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Fig. 12_d

pEXHAM7/E4

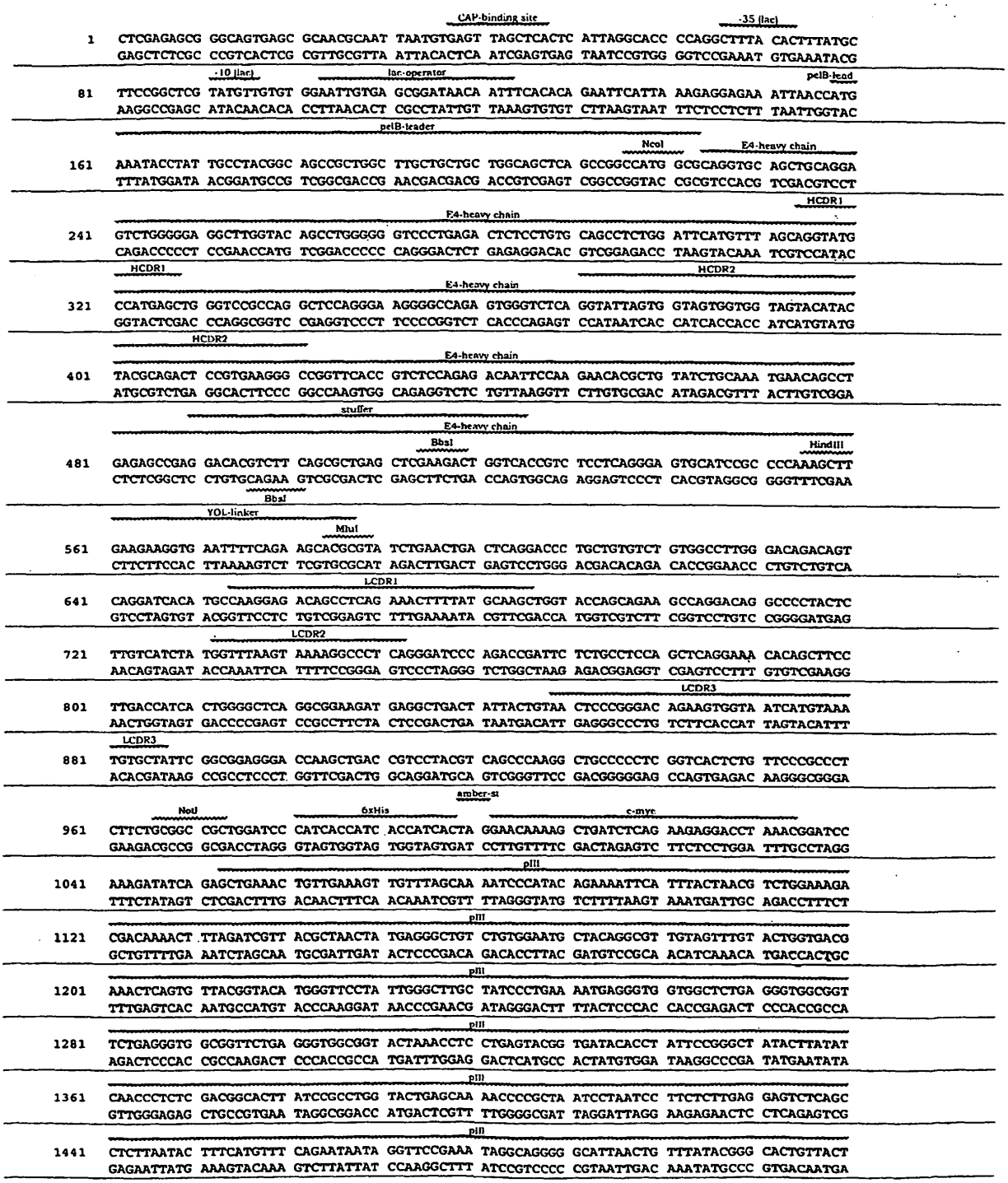

Fig. 13a

pEXHAM7/E4

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                                                                    pIII
1521  CAAGGCACTG ACCCCGTTAA AACTTATTAC CAGTACACTC CTGTATCATC AAAAGCCATG TATGACGCTT ACTGGAACGG
      GTTCCGTGAC TGGGGCAATT TTGAATAATG GTCATGTGAG GACATAGTAG TTTTCGGTAC ATACTGCGAA TGACCTTGCC
                                                                    pIII
1601  TAAATTCAGA GACTGCGCTT TCCATTCTGG CTTTAATGAG GATTTATTTG TTTGTGAATA TCAAGGCCAA TCGTCTGACC
      ATTTAAGTCT CTGACGCGAA AGGTAAGACC GAAATTACTC CTAAATAAAC AAACACTTAT AGTTCCGGTT AGCAGACTGG
                                                                    pIII
1681  TGCCTCAACC TCCTGTCAAT GCTGGCGGCG GCTCTGGTGG TGGTTCTGGT GGCGGCTCTG AGGGTGGTGG CTCTGAGGGT
      ACGGAGTTGG AGGACAGTTA CGACCGCCGC CGAGACCACC ACCAAGACCA CCGCCGAGAC TCCCACCACC GAGACTCCCA
                                                                    pIII
1761  GGCGGTTCTG AGGGTGGCGG CTCTGAGGGA GGCGGTTCCG GTGGTGGCTC TGGTTCCGGT GATTTTGATT ATGAAAAGAT
      CCGCCAAGAC TCCCACCGCC GAGACTCCCT CCGCCAAGGC CACCACCGAG ACCAAGGCCA CTAAAACTAA TACTTTTCTA
                                                                    pIII
1841  GGCAAACGCT AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCGC TACAGTCTGA CGCTAAAGGC AAACTTGATT
      CCGTTTGCGA TTATTCCCCC GATACTGGCT TTTACGGCTA CTTTTGCGCG ATGTCAGACT GCGATTTCCG TTTGAACTAA
                                                                    pIII
1921  CTGTCGCTAC TGATTACGGT GCTGCTATCG ATGGTTTCAT TGGTGACGTT TCCGGCCTTG CTAATGGTAA TGGTGCTACT
      GACAGCGATG ACTAATGCCA CGACGATAGC TACCAAAGTA ACCACTGCAA AGGCCGGAAC GATTACCATT ACCACGATGA
                                                                    pIII
2001  GGTGATTTTG CTGGCTCTAA TTCCCAAATG GCTCAAGTCG GTGACGGTGA TAATTCACCT TTAATGAATA ATTTCCGTCA
      CCACTAAAAC GACCGAGATT AAGGGTTTAC CGAGTTCAGC CACTGCCACT ATTAAGTGGA AATTACTTAT TAAAGGCAGT
                                                                    pIII
2081  ATATTTACCT TCCCTCCCTC AATCGGTTGA ATGTCGCCCT TTTGTCTTTG GCGCTGGTAA ACCATATGAA TTTTCTATTG
      TATAAATGGA AGGGAGGGAG TTAGCCAACT TACAGCGGGA AAACAGAAAC CGCGACCATT TGGTATACTT AAAAGATAAC
                                                                    pIII
2161  ATTGTGACAA AATAAACTTA TTCCGTGGTG TCTTTGCGTT TCTTTTATAT GTTGCCACCT TTATGTATGT ATTTTCTACG
      TAACACTGTT TTATTTGAAT AAGGCACCAC AGAAACGCAA AGAAAATATA CAACGGTGGA AATACATACA TAAAAGATGC
                                                                pIII
2241  TTTGCTAACA TACTGCGTAA TAAGGAGTCT TAATGATCTA GAGGCCTGTG CTAATGATCA GCTAGCTTGA GGCATCAATA
      AAACGATTGT ATGACGCATT ATTCCTCAGA ATTACTAGAT CTCCGGACAC GATTACTAGT CGATCGAACT CCGTAGTTAT
2321  AAACGAAAGG CTCAGTCGAA AGACTGGGCC TTTCGTTTTA TCTGTTGTTT GTCGGTTAAC GTCGACCTGG CGTAATAGCG
      TTTGCTTTCC GAGTCAGCTT TCTGACCCGG AAAGCAAAAT AGACAACAAA CAGCCAATTG CAGCTGGACC GCATTATCGC
2401  AAGAGGCCCG CACCGATCGC CCTTCCCAAC AGTTGCGCAG CCTGAATGGC GAATGGGACG CGCCCTGTAG CGGCGCATTA
      TTCTCCGGGC GTGGCTAGCG GGAAGGGTTG TCAACGCGTC GGACTTACCG CTTACCCTGC GCGGGACATC GCCGCGTAAT
2481  AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT
      TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC GCGGGATCGC GGGCGAGGAA AGCGAAAGAA
2561  CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC CGATTTAGTG
      GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG GCTAAATCAC
2641  CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT
      GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAATCCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA
2721  CGCCCTTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGT
      GCGGGAAACT GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCA
2801  CTATTCTTTT GATTTATAAG GGATTTTGCC GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG
      GATAAGAAAA CTAAATATTC CCTAAAACGG CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC
2881  CGAATTTTAA CAAAATATTA ACGCTTACAA TTTAGGTGGC ACTTTTCGGG GAAATGTGCG CGGAACCCCT ATTTGTTTAT
      GCTTAAAATT GTTTTATAAT TGCGAATGTT AAATCCACCG TGAAAAGCCC CTTTACACGC GCCTTGGGGA TAAACAAATA
2961  TTTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA ATAACCCTGA TAAATGCTTC AATAATATTG AAAAAGGAAG
      AAAAGATTTA TGTAAGTTTA TACATAGGCG AGTACTCTGT TATTGGGACT ATTTACGAAG TTATTATAAC TTTTTCCTTC
                                                                    bla
3041  AGTATGAGTA TTCAACATTT CCGTGTCGCC CTTATTCCCT TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA
      TCATACTCAT AAGTTGTAAA GGCACAGCGG GAATAAGGGA AAAAACGCCG TAAAACGGAA GGACAAAAAC GAGTGGGTCT
                                                                    bla
3121  AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA
      TTGCGACCAC TTTCATTTTC TACGACTTCT AGTCAACCCA CGTGCTCACC CAATGTAGCT TGACCTAGAG TTGTCGCCAT
                                                                    bla
3201  AGATCCTTGA GAGTTTTCGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC TGCTATGTGG CGCGGTATTA
      TCTAGGAACT CTCAAAAGCG GGGCTTCTTG CAAAAGGTTA CTACTCGTGA AAATTTCAAG ACGATACACC GCGCCATAAT
                                                                    bla
3281  TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT
      AGGGCATAAC TGCGGCCCGT TCTCGTTGAG CCAGCGGCGT ATGTGATAAG AGTCTTACTG AACCAACTCA TGAGTGGTCA
                                                                    bla
3361  CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG
      GTGTCTTTTC GTAGAATGCC TACCGTACTG TCATTCTCTT AATACGTCAC GACGGTATTG GTACTCACTA TTGTGACGCC
                                                                    bla
3441  CCAACTTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC
      GGTTGAATGA AGACTGTTGC TAGCCTCCTG GCTTCCTCGA TTGGCGAAAA AACGTGTTGT ACCCCCTAGT ACATTGAGCG
```

Fig. 13b

pEXHAM7/E4

```
                                          bla
3521  CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC
      GAACTAGCAA CCCTTGGCCT CGACTTACTT CGGTATGGTT TGCTGCTCGC ACTGTGGTGC TACGGACATC GTTACCGTTG
                                          bla
3601  AACGTTGCGC AAACTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT AGACTGGATG GAGGCGGATA
      TTGCAACGCG TTTGATAATT GACCGCTTGA TGAATGAGAT CGAAGGGCCG TTGTTAATTA TCTGACCTAC CTCCGCCTAT
                                          bla
3681  AAGTTGCAGG ACCACTTCTG CGCTCGGCCC TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG
      TTCAACGTCC TGGTGAAGAC GCGAGCCGGG AAGGCCGACC GACCAAATAA CGACTATTTA GACCTCGGCC ACTCGCACCC
                                          bla
3761  TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG GGAGTCAGGC
      AGAGCGCCAT AGTAACGTCG TGACCCCGGT CTACCATTCG GGAGGGCATA GCATCAATAG ATGTGCTGCC CCTCAGTCCG
                                   bla
3841  AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAACTGTCA GACCAAGTTT
      TTGATACCTA CTTGCTTTAT CTGTCTAGCG ACTCTATCCA CGGAGTGACT AATTCGTAAC CATTGACAGT CTGGTTCAAA
3921  ACTCATATAT ACTTTAGATT GATTTAAAAC TTCATTTTTA ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC
      TGAGTATATA TGAAATCTAA CTAAATTTTG AAGTAAAAAT TAAATTTTCC TAGATCCACT TCTAGGAAAA ACTATTAGAG
4001  ATGACCAAAA TCCCTTAACG TGAGTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA
      TACTGGTTTT AGGGAATTGC ACTCAAAAGC AAGGTGACTC GCAGTCTGGG GCATCTTTTC TAGTTTCCTA GAAGAACTCT
4081  TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC TACCAGCGGT GGTTTGTTTG CCGGATCAAG
      AGGAAAAAAA GACGCGCATT AGACGACGAA CGTTTGTTTT TTTGGTGGCG ATGGTCGCCA CCAAACAAAC GGCCTAGTTC
4161  AGCTACCAAC TCTTTTTCCG AAGGTAACTG GCTTCAGCAG AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG
      TCGATGGTTG AGAAAAAGGC TTCCATTGAC CGAAGTCGTC TCGCGTCTAT GGTTTATGAC AGGAAGATCA CATCGGCATC
4241  TTAGGCCACC ACTTCAAGAA CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG
      AATCCGGTGG TGAAGTTCTT GAGACATCGT GGCGGATGTA TGGAGCGAGA CGATTAGGAC AATGGTCACC GACGACGGTC
4321  TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA GCGGTCGGGC TGAACGGGGG
      ACCGCTATTC AGCACAGAAT GGCCCAACCT GAGTTCTGCT ATCAATGGCC TATTCCGCGT CGCCAGCCCG ACTTGCCCCC
4401  GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA CGACCTACAC CGAACTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC
      CAAGCACGTG TGTCGGGTCG AACCTCGCTT GCTGGATGTG GCTTGACTCT ATGGATGTCG CACTCGATAC TCTTTCGCGG
4481  ACGCTTCCCG AAGGGAGAAA GGCGGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC
      TGCGAAGGGC TTCCCTCTTT CCGCCTGTCC ATAGGCCATT CGCCGTCCCA GCCTTGTCCT CTCGCGTGCT CCCTCGAAGG
4561  AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GACTTGAGCG TCGATTTTTG TGATGCTCGT
      TCCCCCTTTG CGGACCATAG AAATATCAGG ACAGCCCAAA GCGGTGGAGA CTGAACTCGC AGCTAAAAAC ACTACGAGCA
4641  CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC
      GTCCCCCCGC CTCGGATACC TTTTTGCGGT CGTTGCGCCG GAAAAATGCC AAGGACCGGA AAACGACCGG AAAACGAGTG
4721  ATGTTCTTTC CTGCGTTATC CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGCAG
      TACAAGAAAG GACGCAATAG GGGACTAAGA CACCTATTGG CATAATGGCG GAAACTCACT CGACTATGGC GAGCGGCGTC
4801  CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC CAATACGCAA ACCGCCTCTC CCCGCGCGTT
      GGCTTGCTGG CTCGCGTCGC TCAGTCACTC GCTCCTTCGC CTTCTCGCGG GTTATGCGTT TGGCGGAGAG GGGCGCGCAA
4881  GGCCGATTCA TTAATGCAGG TATCACGAGG CCCTTTCGTC CTCAC
      CCGGCTAAGT AATTACGTCC ATAGTGCTCC GGGAAAGCAG GAGTG
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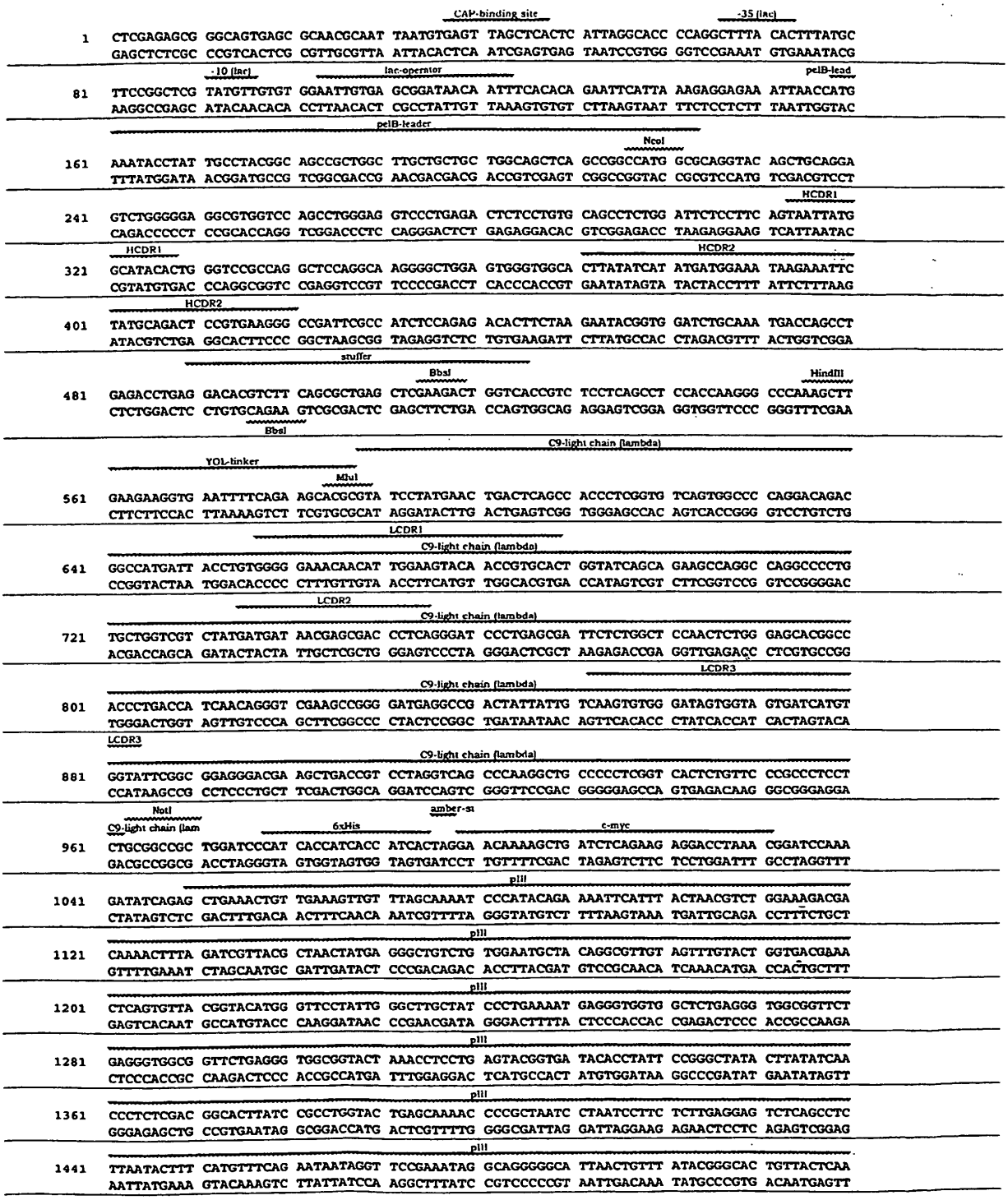

Fig. 14a

pEXHAM7/C9

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                                                                pIII
1521  GGCACTGACC CCGTTAAAAC TTATTACCAG TACACTCCTG TATCATCAAA AGCCATGTAT GACGCTTACT GGAACGGTAA
      CCGTGACTGG GGCAATTTTG AATAATGGTC ATGTGAGGAC ATAGTAGTTT TCGGTACATA CTGCGAATGA CCTTGCCATT
                                                                pIII
1601  ATTCAGAGAC TGCGCTTTCC ATTCTGGCTT TAATGAGGAT TTATTTGTTT GTGAATATCA AGGCCAATCG TCTGACCTGC
      TAAGTCTCTG ACGCGAAAGG TAAGACCGAA ATTACTCCTA AATAAACAAA CACTTATAGT TCCGGTTAGC AGACTGGACG
                                                                pIII
1681  CTCAACCTCC TGTCAATGCT GGCGGCGGCT CTGGTGGTGG TTCTGGTGGC GGCTCTGAGG GTGGTGGCTC TGAGGGTGGC
      GAGTTGGAGG ACAGTTACGA CCGCCGCCGA GACCACCACC AAGACCACCG CCGAGACTCC CACCACCGAG ACTCCCACCG
                                                                pIII
1761  GGTTCTGAGG GTGGCGGCTC TGAGGGAGGC GGTTCCGGTG GTGGCTCTGG TTCCGGTGAT TTTGATTATG AAAAGATGGC
      CCAAGACTCC CACCGCCGAG ACTCCCTCCG CCAAGGCCAC CACCGAGACC AAGGCCACTA AAACTAATAC TTTTCTACCG
                                                                pIII
1841  AAACGCTAAT AAGGGGGCTA TGACCGAAAA TGCCGATGAA AACGCGCTAC AGTCTGACGC TAAAGGCAAA CTTGATTCTG
      TTTGCGATTA TTCCCCCGAT ACTGGCTTTT ACGGCTACTT TTGCGCGATG TCAGACTGCG ATTTCCGTTT GAACTAAGAC
                                                                pIII
1921  TCGCTACTGA TTACGGTGCT GCTATCGATG GTTTCATTGG TGACGTTTCC GGCCTTGCTA ATGGTAATGG TGCTACTGGT
      AGCGATGACT AATGCCACGA CGATAGCTAC CAAAGTAACC ACTGCAAAGG CCGGAACGAT TACCATTACC ACGATGACCA
                                                                pIII
2001  GATTTTGCTG GCTCTAATTC CCAAATGGCT CAAGTCGGTG ACGGTGATAA TTCACCTTTA ATGAATAATT TCCGTCAATA
      CTAAAACGAC CGAGATTAAG GGTTTACCGA GTTCAGCCAC TGCCACTATT AAGTGGAAAT TACTTATTAA AGGCAGTTAT
                                                                pIII
2081  TTTACCTTCC CTCCCTCAAT CGGTTGAATG TCGCCCTTTT GTCTTTGGCG CTGGTAAACC ATATGAATTT TCTATTGATT
      AAATGGAAGG GAGGGAGTTA GCCAACTTAC AGCGGGAAAA CAGAAACCGC GACCATTTGG TATACTTAAA AGATAACTAA
                                                                pIII
2161  GTGACAAAAT AAACTTATTC CGTGGTGTCT TTGCGTTTCT TTTATATGTT GCCACCTTTA TGTATGTATT TTCTACGTTT
      CACTGTTTTA TTTGAATAAG GCACCACAGA AACGCAAAGA AAATATACAA CGGTGGAAAT ACATACATAA AAGATGCAAA
                                pIII
2241  GCTAACATAC TGCGTAATAA GGAGTCTTAA TGATCTAGAG GCCTGTGCTA ATGATCAGCT AGCTTGAGGC ATCAATAAAA
      CGATTGTATG ACGCATTATT CCTCAGAATT ACTAGATCTC CGGACACGAT TACTAGTCGA TCGAACTCCG TAGTTATTTT
2321  CGAAAGGCTC AGTCGAAAGA CTGGGCCTTT CGTTTTATCT GTTGTTTGTC GGTTAACGTC GACCTGGCGT AATAGCGAAG
      GCTTTCCGAG TCAGCTTTCT GACCCGGAAA GCAAAATAGA CAACAAACAG CCAATTGCAG CTGGACCGCA TTATCGCTTC
2401  AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGCAGCCT GAATGGCGAA TGGGACGCGC CCTGTAGCGG CGCATTAAGC
      TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCGTCGGA CTTACCGCTT ACCCTGCGCG GGACATCGCC GCGTAATTCG
2481  GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC
      CGCCGCCCAC ACCACCAATG CGCGTCGCAC TGGCGATGTG AACGGTCGCG GGATCGCGGG CGAGGAAAGC GAAAGAAGGG
2561  TTCCTTTCTC GCCACGTTCG CCGGCTTTCC CCGTCAAGCT CTAAATCGGG GGCTCCCTTT AGGGTTCCGA TTTAGTGCTT
      AAGGAAAGAG CGGTGCAAGC GGCCGAAAGG GGCAGTTCGA GATTTAGCCC CCGAGGGAAA TCCCAAGGCT AAATCACGAA
2641  TACGGCACCT CGACCCCAAA AAACTTGATT AGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC GGTTTTTCGC
      ATGCCGTGGA GCTGGGGTTT TTTGAACTAA TCCCACTACC AAGTGCATCA CCCGGTAGCG GGACTATCTG CCAAAAAGCG
2721  CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC TGGAACAACA CTCAACCCTA TCTCGGTCTA
      GGAAACTGCA ACCTCAGGTG CAAGAAATTA TCACCTGAGA ACAAGGTTTG ACCTTGTTGT GAGTTGGGAT AGAGCCAGAT
2801  TTCTTTTGAT TTATAAGGGA TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA TTTAACGCGA
      AAGAAAACTA AATATTCCCT AAAACGGCTA AAGCCGGATA ACCAATTTTT TACTCGACTA AATTGTTTTT AAATTGCGCT
2881  ATTTTAACAA AATATTAACG CTTACAATTT AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT
      TAAAATTGTT TTATAATTGC GAATGTTAAA TCCACCGTGA AAAGCCCCTT TACACGCGCC TTGGGGATAA ACAAATAAAA
2961  TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT
      AGATTTATGT AAGTTTATAC ATAGGCGAGT ACTCTGTTAT TGGGACTATT TACGAAGTTA TTATAACTTT TTCCTTCTCA
                                                                bla
3041  ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC
      TACTCATAAG TTGTAAAGGC ACAGCGGGAA TAAGGGAAAA AACGCCGTAA AACGGAAGGA CAAAAACGAG TGGGTCTTTG
                                                                bla
3121  GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA
      CGACCACTTT CATTTTCTAC GACTTCTAGT CAACCCACGT GCTCACCCAA TGTAGCTTGA CCTAGAGTTG TCGCCATTCT
                                                                bla
3201  TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC
      AGGAACTCTC AAAAGCGGGG CTTCTTGCAA AAGGTTACTA CTCGTGAAAA TTTCAAGACG ATACACCGCG CCATAATAGG
                                                                bla
3281  CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC
      GCATAACTGC GGCCCGTTCT CGTTGAGCCA GCGGCGTATG TGATAAGAGT CTTACTGAAC CAACTCATGA GTGGTCAGTG
                                                                bla
3361  AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA
      TCTTTTCGTA GAATGCCTAC CGTACTGTCA TTCTCTTAAT ACGTCACGAC GGTATTGGTA CTCACTATTG TGACGCCGGT
                                                                bla
3441  ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT
      TGAATGAAGA CTGTTGCTAG CCTCCTGGCT TCCTCGATTG GCGAAAAAAC GTGTTGTACC CCCTAGTACA TTGAGCGGAA
```

Fig. 14b

pEXHAM7/C9

bla

3521 GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGTAGCAA TGGCAACAAC
CTAGCAACCC TTGGCCTCGA CTTACTTCGG TATGGTTTGC TGCTCGCACT GTGGTGCTAC GGACATCGTT ACCGTTGTTG

bla

3601 GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG
CAACGCGTTT GATAATTGAC CGCTTGATGA ATGAGATCGA AGGGCCGTTG TTAATTATCT GACCTACCTC CGCCTATTTC

bla

3681 TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT
AACGTCCTGG TGAAGACGCG AGCCGGGAAG GCCGACCGAC CAAATAACGA CTATTTAGAC CTCGGCCACT CGCACCCAGA

bla

3761 CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC
GCGCCATAGT AACGTCGTGA CCCCGGTCTA CCATTCGGGA GGGCATAGCA TCAATAGATG TGCTGCCCCT CAGTCCGTTG

bla

3841 TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT
ATACCTACTT GCTTTATCTG TCTAGCGACT CTATCCACGG AGTGACTAAT TCGTAACCAT TGACAGTCTG GTTCAAATGA

3921 CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG
GTATATATGA AATCTAACTA AATTTTGAAG TAAAAATTAA ATTTTCCTAG ATCCACTTCT AGGAAAAACT ATTAGAGTAC

4001 ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC
TGGTTTTAGG GAATTGCACT CAAAAGCAAG GTGACTCGCA GTCTGGGGCA TCTTTTCTAG TTTCCTAGAA GAACTCTAGG

4081 TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC
AAAAAAAGAC GCGCATTAGA CGACGAACGT TTGTTTTTTT GGTGGCGATG GTCGCCACCA AACAAACGGC CTAGTTCTCG

4161 TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA
ATGGTTGAGA AAAAGGCTTC CATTGACCGA AGTCGTCTCG CGTCTATGGT TTATGACAGG AAGATCACAT CGGCATCAAT

4241 GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG
CCGGTGGTGA AGTTCTTGAG ACATCGTGGC GGATGTATGG AGCGAGACGA TTAGGACAAT GGTCACCGAC GACGGTCACC

4321 CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT
GCTATTCAGC ACAGAATGGC CCAACCTGAG TTCTGCTATC AATGGCCTAT TCCGCGTCGC CAGCCCGACT TGCCCCCCAA

4401 CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG
GCACGTGTGT CGGGTCGAAC CTCGCTTGCT GGATGTGGCT TGACTCTATG GATGTCGCAC TCGATACTCT TTCGCGGTGC

4481 CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG
GAAGGGCTTC CCTCTTTCCG CCTGTCCATA GGCCATTCGC CGTCCCAGCC TTGTCCTCTC GCGTGCTCCC TCGAAGGTCC

4561 GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG
CCCTTTGCGG ACCATAGAAA TATCAGGACA GCCCAAAGCG GTGGAGACTG AACTCGCAGC TAAAAACACT ACGAGCAGTC

4641 GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG
CCCCCGCCTC GGATACCTTT TTGCGGTCGT TGCGCCGGAA AAATGCCAAG GACCGGAAAA CGACCGGAAA ACGAGTGTAC

4721 TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG
AAGAAAGGAC GCAATAGGGG ACTAAGACAC CTATTGGCAT AATGGCGGAA ACTCACTCGA CTATGGCGAG CGGCGTCGGC

4801 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC
TTGCTGGCTC GCGTCGCTCA GTCACTCGCT CCTTCGCCTT CTCGCGGGTT ATGCGTTTGG CGGAGAGGGG CGCGCAACCG

4881 CGATTCATTA ATGCAGGTAT CACGAGGCCC TTTCGTCCTC AC
GCTAAGTAAT TACGTCCATA GTGCTCCGGG AAAGCAGGAG TG

Fig. 14c

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	用于抑制血小板聚集的人源抗体		
公开(公告)号	EP1300419B1	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 C12N1/15 C12N1/19 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	Espacenet		

摘要(译)

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的活化状态而有效。


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tggccccagg acagacggcc aggattacct gtgggggaaa caacattgga agtaaaagtg   540
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gtagtagtga tcatgtggta ttcggcggag ggaccaagct gaccgtccta ggtcagccca   780
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