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(54) Antibody of human origin for inhibiting thrombocyte aggregation

(57) The present invention is directed to an antibody or derivative thereof of human origin for inhibiting platelet aggregation, characterized in that it is effective by substantially exclusive binding to the activated state of platelet integrin receptor GPIIb/IIIa.

EP 1 300 419 A1

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) :3240-)
- 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 proteinases. Thus, e.g., the antibody might be linked to an effector molecule having a conformation suitable for biological activity or selective binding to a solid support, a biologically active substance (e.g. a cytokine or growth hormone), a chemical agent, a peptide, a protein or a drug.

[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, pBlue-Script, 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] The step of providing a library may comprise the following steps:

- isolating whole RNA from human donors;
- isolating mRNA contained in the whole RNA coding for antibody polypeptides;

- generation of cDNA; and

- deriving DNA molecules coding for single chain fragments from cDNA molecules coding for antibody polypeptides.

[0027] By this, a "natural library", based on the antibody population of the donors, can be obtained

[0028] 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.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] 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.

[0034] 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, intraperitoneally 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.).

[0035] 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).

[0036] 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.

[0037] 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

[0038] In the following, examples for the production of human scFv antibodies specific for activated platelet integrin receptor GPIIb/IIIa will be given.

General strategy

[0039] 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

[0040] 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

[0041] 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

[0042] Heavy chain fragments are digested with *Nco*I and *Hind*III, 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.

[0043] 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

[0044] 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).

Screening the library for scFv's binding activated platelets

[0045] 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:

[0046] 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:

[0047] 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:

[0048] 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:

[0049] 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

[0050] Depletion of scFv's binding non activated GPIIb/IIIa: 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:

[0051] CHO cells presenting active GPIIb/IIIa (Cl3 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:

[0052] 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:

[0053] 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.

[0054] 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

[0055] 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

[0056] FACS-analysis is done using a FACSCalibur device (Becton Dickinson).

Analysis of activation specificity:

[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 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:

[0058] 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

[0059] 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).

[0060] 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.

[0061] 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, *Hin*dIII and *Mlu*I, *Not*I respectively) are indicated.

[0062] A clone encoding MB9 has been deposited under DSM 14491 (XLIblue(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	++	++	+

Table 1: (continued)

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

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

Origin of human scFv master frameworks

[0063] 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

[0064] 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.
[0065] 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).
[0066] 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

[0067] 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.
[0068] 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.

[0069] 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

[0070] Packaging of synthetic libraries is done as described for the natural library (example 1).

Screening of the synthetic library

[0071] 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

[0072] 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 GATYTS**RSD**VPDQTS FDY	2	VHCDR3_ev2/for/cut
SA3	CAR DDLAYC**RGD**CSGRFA 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 ELEAYC**RGD**CYPPYYG MDV	1	VHCDR3_ev3/for/cut
SA10	CAR DLFRG**RGD**YGDYG MDV	1	VHCDR3_ev2/for/cut
SA11	CAR TYYYDS**RTD**RRPPHA FDI	1	VHCDR3_ev3/for/cut

[0073] 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).

[0074] The selected clones probably interact exactly with the fibrinogen binding site of the GPIIb/IIa receptor but with an affinity similar or lower than fibrinogen. The affinity has been enhanced by mutation within the VH and/or the VL-domain of the scFv antibody fragment or the exchange of the whole VL domain ("chain shuffling).

SEQUENCE LISTING

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<223> Description of the Artificial Sequence: Plasmid

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<223> Description of the Artificial Sequence: Plasmid

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<210> 10
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<210> 13
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<210> 14
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<400> 16
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oligonucleotide

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oligonucleotide

<400> 21
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<210> 22
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oligonucleotide

<400> 22
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<210> 23
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oligonucleotide

<400> 23
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<210> 24
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<400> 24
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<210> 25
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<400> 25
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<400> 26
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<400> 27
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<400> 28
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<210> 29
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<400> 29
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<210> 30
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<400> 30
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<210> 31
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<210> 32
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<210> 33
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<210> 34
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<210> 35
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<400> 35
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<210> 36
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<400> 36
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<210> 37
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<400> 37
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<210> 38
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<400> 38
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<210> 40
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<210> 41
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<210> 42
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<210> 43
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<210> 44
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<210> 45
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<210> 46
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<210> 47
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<210> 48
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<210> 49
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<400> 49
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<210> 50
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<210> 51
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<210> 52
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<210> 53
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<400> 53
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<210> 54
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Oligonucleotide

<400> 54
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<210> 55
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<400> 55
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<210> 56
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<400> 56
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<210> 57
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<400> 57
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<210> 58
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<400> 58
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<210> 59
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<400> 59
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<210> 60
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<400> 60
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<210> 61
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<210> 62
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<400> 62
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<210> 63
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<400> 63
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<210> 64

<211> 21
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<210> 65
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<210> 66
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<210> 67
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<210> 68
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<210> 69
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Oligonucleotide

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<210> 70
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<210> 71
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Oligonucleotide

<400> 71
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<210> 72
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oligonucleotide

<400> 72
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<210> 73
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<400> 73
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<210> 74

<211> 36
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oligonucleotide

<400> 74
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<210> 75
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oligonucleotide

<400> 75
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<210> 76
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oligonucleotide

<400> 76
tggacgccca tggcgcagat gcagctggta cagtct 36

<210> 77
<211> 36
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oligonucleotide

<400> 77
tggacgccca tggcgcagat caccttgaag gagtct 36

<210> 78
<211> 36
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oligonucleotide

<400> 78
tggacgccca tggcgcaggt caccttgaag gagtct 36

<210> 79
<211> 36
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oligonucleotide

<400> 79
tggacgccca tggcggaagt gcagctggtg gagtct 36

<210> 80
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oligonucleotide

<400> 80
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<210> 81
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oligonucleotide

<400> 81
tggacgccca tggcggaggt gcagctgttg gagtct 36

<210> 82
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oligonucleotide

<400> 82
tggacgccca tggcgcaggt gcagctgcag gagtcg 36

<210> 83
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oligonucleotide

<400> 83
tggacgccca tggcgcagct gcagctgcag gagtcg 36

<210> 84
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oligonucleotide

<400> 84
tggacgccca tggcgcaggt gcagctacag cagtgg 36

<210> 85
<211> 36
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oligonucleotide

<400> 85
tggacgccca tggcggaagt gcagctggtg cagtct 36

<210> 86
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<223> Description of Artificial Sequence:

oligonucleotide

<400> 86
tggacgccca tggcgcaggt acagctgcag cagtca 36

<210> 87
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oligonucleotide

<400> 87
tggacgccca tggcgcaggt gcagctggtg caatct 36

<210> 88
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oligonucleotide

<400> 88
tgggaaaagc ttaagggttg gggcggatgc act 33

<210> 89
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Oligonucleotide

<400> 89
cctacagaac gcgtacagtc tgtgctgacg cagcca 36

<210> 90
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Oligonucleotide

<400> 90
cctacagaac gcgtacagtc tgtgctgacg cagccg 36

<210> 91
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Oligonucleotide

<400> 91
cctacagaac gcgtacagtc tgccctgact cagcct 36

<210> 92
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Oligonucleotide

<400> 92
cctacagaac gcgtatccta tgagctgaca cagcca 36

<210> 93
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Oligonucleotide

<400> 93
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<210> 94
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Oligonucleotide

<400> 94
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<210> 95
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Oligonucleotide

<400> 95
cctacagaac gcgtatccta tgagctgaca cagcta 36

<210> 96
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Oligonucleotide

<400> 96
cctacagaac gcgtatccta tgagctgatg cagcca 36

<210> 97
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Oligonucleotide

<400> 97
cctacagaac gcgtactgcc tgtgctgact cagccc 36

<210> 98
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Oligonucleotide

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<210> 99
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Oligonucleotide

<400> 99
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<210> 100
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Oligonucleotide

<400> 100
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<210> 101
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Oligonucleotide

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<210> 102
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Oligonucleotide

<400> 102
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<210> 103
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Oligonucleotide

<400> 103
cctacagaac gcgtacagac tgtggtgact caggag 36

<210> 104
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Oligonucleotide

<400> 104
cctacagaac gcgtacaggc tgtggtgact caggag 36

<210> 105
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<223> Description of Artificial Sequence:
Oligonucleotide

<400> 105
cctacagaac gcgtacagac tgtggtgacc caggag 36

<210> 106
<211> 36
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Met Asp Val

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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 of blood thrombocytes , comprising the following steps:

- providing a library of nucleic acids encoding for sequences of antibodies;
- 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.

9. The process according to claim 8, **characterised in that** the step of providing a library comprises the following steps:

- isolating whole RNA from human donors;
- isolating mRNA contained in the whole RNA;
- generation of cDNA;
- deriving DNA molecules coding for single chain fragments from cDNA molecules coding for antibody polypeptides;

10. The process according to claim 8, **characterised in that** 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.

11. The process according to claim 10, **characterised in that** the region into which the at least one randomised nucleotide sequence is introduced, is the CDR3 region.

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

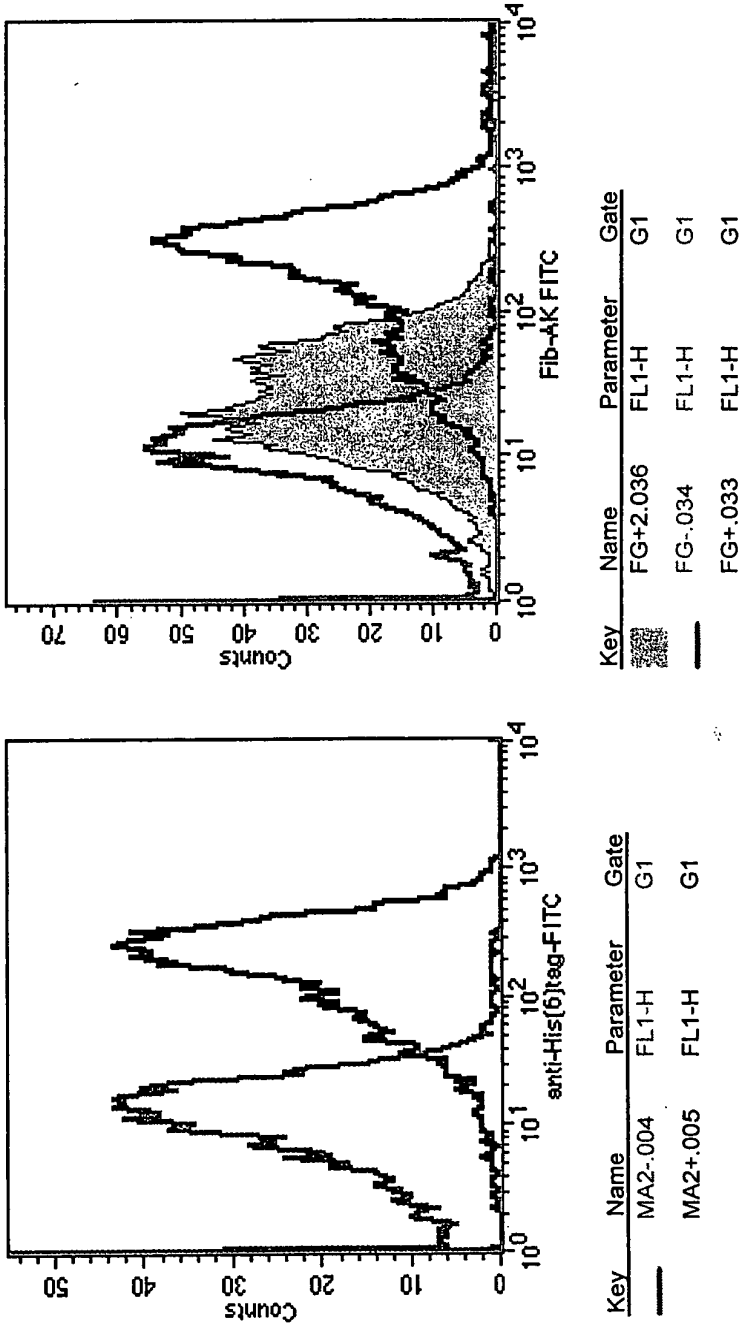
13. Expression vector containing the DNA sequence according to claim 12.

14. Cell line containing the DNA sequence according to claim 12 or the expression vector according to claim 13.

15. Pharmaceutical composition containing the antibody according to any of claim 1-7 , the DNA sequence according to claim 12 or the expression vector according to claim 13.

16. Use of the antibody according to any of claim 1-7 , the DNA sequence according to claim 12 or the expression vector according to claim 13 for preparing a pharmaceutical composition for blocking the platelet integrin receptor on thrombocytes.

17. Use of the antibody according to any of claim 1-7 , the DNA sequence according to claim 12 or the expression vector according to claim 13 for preparing a diagnostic composition for determining the number of activated thrombocytes.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Key Name Parameter Gate
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FG+2.036 FL1-H G1
FG-.034 FL1-H G1
FG+.033 FL1-H G1

Fig. 1

SEQ No. 1

NcoI

```
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 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
*Hin*dIII
361 tcaccgtctc ctcagcctcc accaagggcc caaagcttga agaaggtgaa ttttcagaag
*Mlu*I
421 cacgcgtatc ctatgaactg actcagccac cctcggtgtc agtggcccca ggacagacgg
481 ccatgattac ctgtggggga 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)

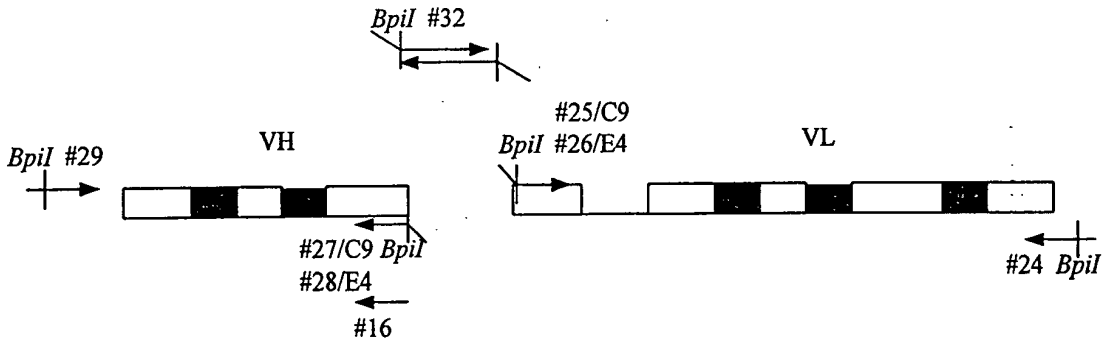
*Nco*I
1 ccatggcgca ggtgcagctg caggagtctg ggggaggctt ggtacagcct gggggtccc
61 tgagactctc ctgtgcagcc tctggattca tgtttagcag gtatgccatg agctgggtcc
121 gccaggctcc agggaagggg ccagagtggg tctcaggtat tagtggtagt ggtggtagta
181 catactacgc agactccgtg aagggccggt tcaccgtctc cagagacaat tccaagaaca
241 cgctgtatct gcaaatgaac agcctgagag ccgaggacac ggccgtatat tactgtgcga
301 aagatctggg ctactatggt tcggggagcc aacccttga gtactggggc cagggaactc
*Hin*dIII
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, *Hin*dIII 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.

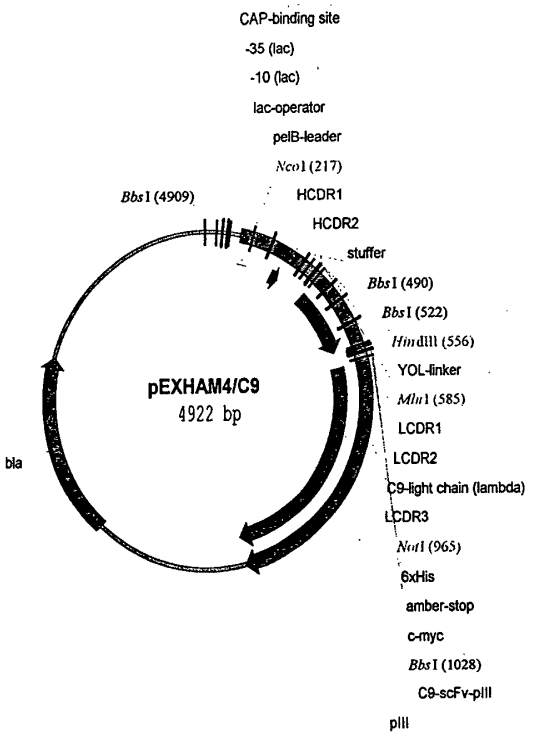
vector construction	library construction
C9/VHCDR1.2/back/cut (#27): TAC TAC **GAA GAC** GTG TCC TCA GGT CTC AGG CTG GTC E4/VHCDR1.2/back/cut (#28): TAC TAC **GAA GAC** GTG TCC TCG GCT CTC AGG CTG TTC VH/for/cut (#29): AAT GCA GGT ATC ACG AGG CCC TTT **CGT CTT C** VL/back/cut (#24): CAG CTC TGA TAT CTT TGG ATC CGT TTA **GGT CTT C**TT 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



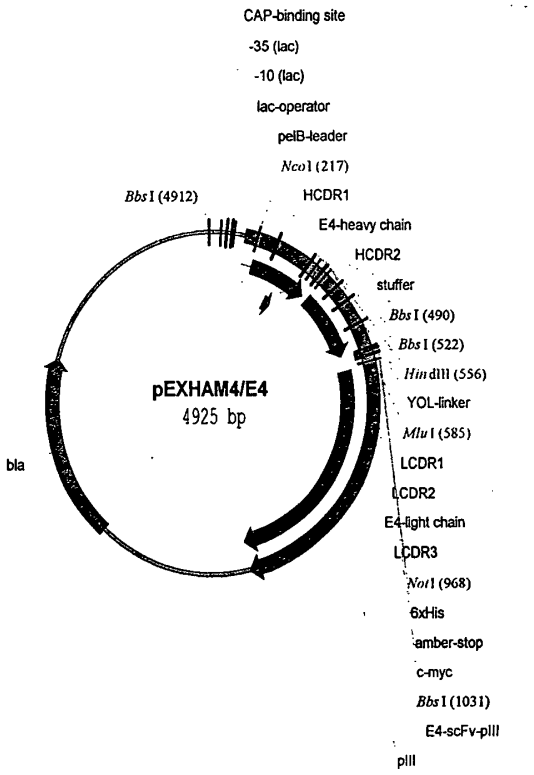
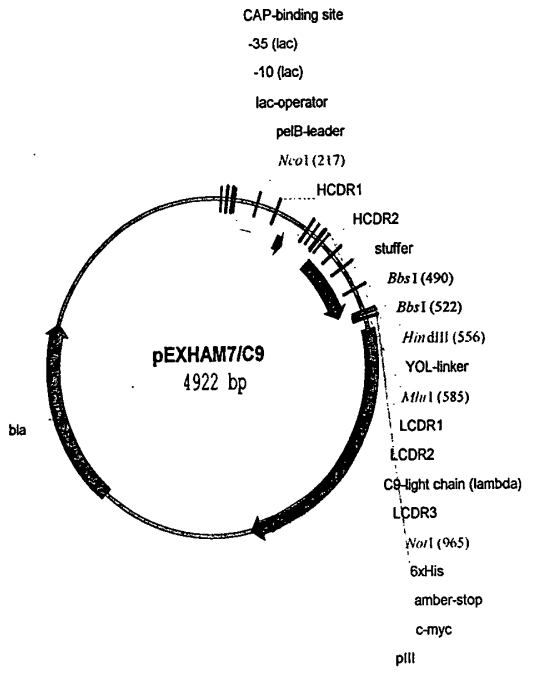


Figure 6: Vectormaps of pEXHAM4/C9 and pEXHAM4/E4



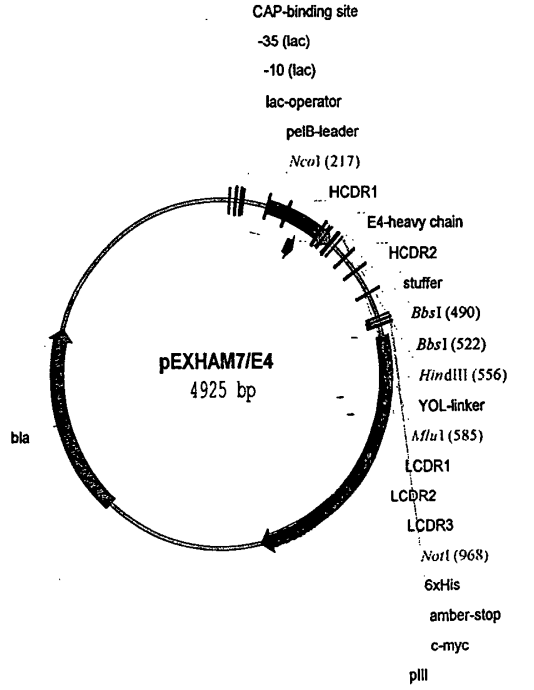


Figure 7: Vectormaps of pEXHAM7/C9 and pEXHAM7/E4

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

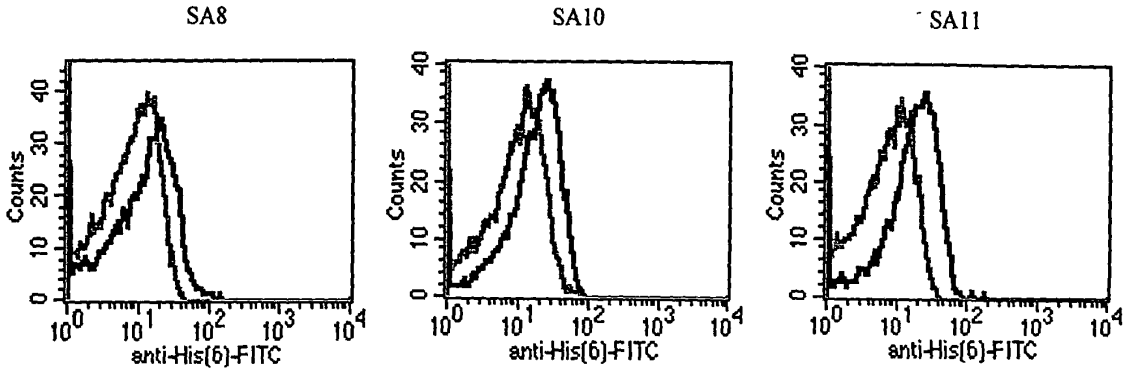
heavy chain primer		light chain primer	
VH-1a.	CAG GTG CAG CTG GTG CAG TCT 22	Vλ-1a.	CAG TCT GTG CTG ACG CAG CCA 39
VH-1b.	CAG GTC CAG CTT GTG CAG TCT	Vλ-1b.	CAG TCT GTG CTG ACG CAG CCG
VH-1c.	CAG GTC CAG CTG GTA CAG TCT	Vλ-2	CAG TCT GCC CTG ACT CAG CCT
VH-1d.	GAG GTC CAG CTG GTA CAG TCT	Vλ-3a.	TCC TAT 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 76

Fig. 8

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

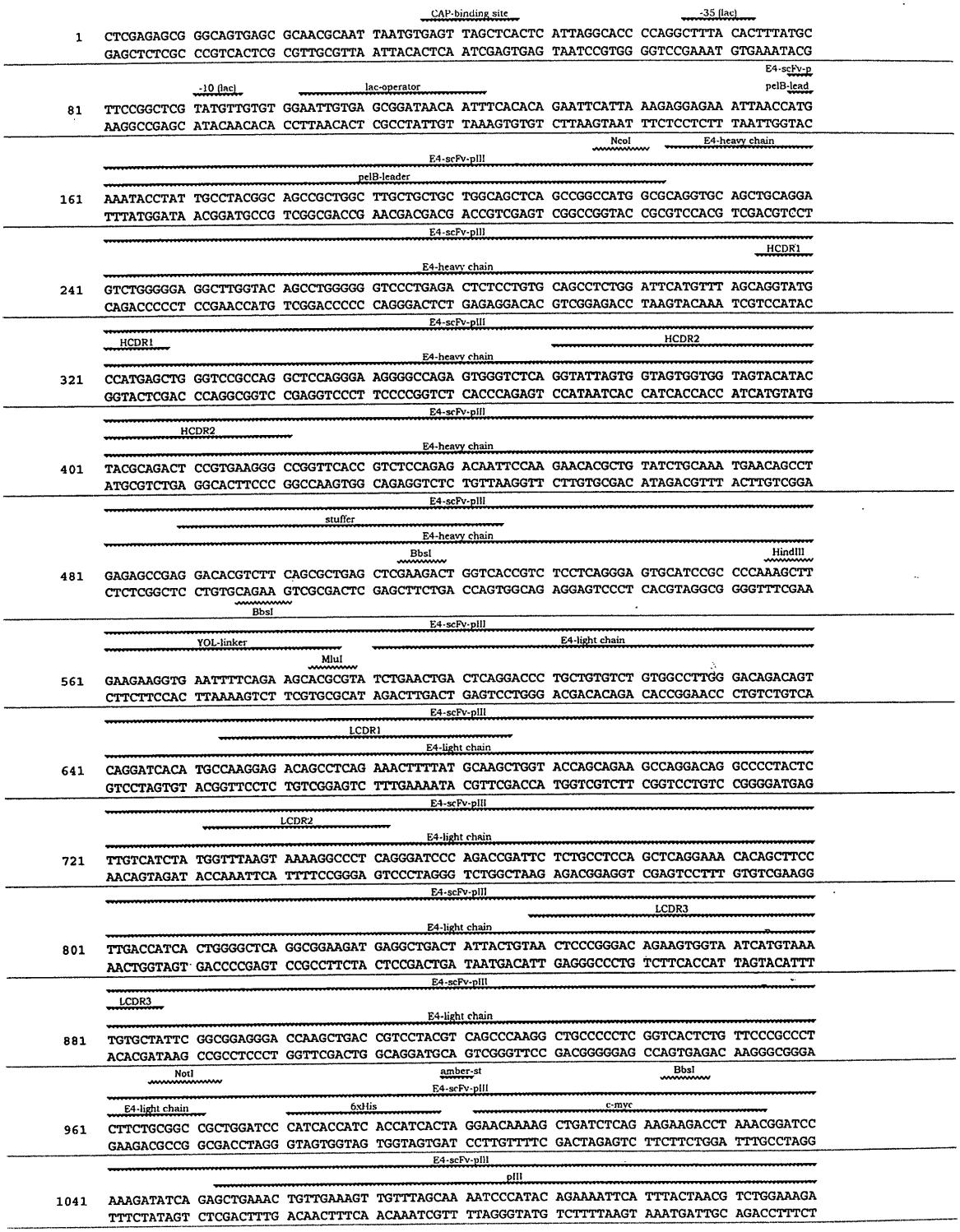
heavy chain primer		light chain primer	
	NcoI		MluI
VH-1a.	TGG ACG CCC ATG GCG CAG GTG CAG CTG GTG CAG TCT 77	Vλ-1a.	CCT ACA GAA CGC GTA CAG TCT GTG CTG ACG CAG CCA 94
VH-1b.	TGG ACG CCC ATG GCG CAG GTC CAG CTT GTG CAG TCT	Vλ-1b.	CCT ACA GAA CGC GTA CAG TCT GTG CTG ACG CAG CCG
VH-1c.	TGG ACG CCC ATG 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
		Vλ-8	CCT ACA GAA CGC GTA CAG ACT GTG GTG ACC CAG GAG
	HindIII	Vλ-9	CCT ACA GAA CGC GTA CAG CCT GTG CTG ACT CAG CCA
IgM	TGG GAA AAG CTT AAG GGT TGG GGC GGA TGC ACT 93	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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E4-scFv-p
-10 (lac)
lac-operator
pelB-lead
NcoI
E4-heavy chain
E4-scFv-pIII
pelB-leader
HCDR1
HCDR2
stuffer
BbsI
HindIII
YOL-linker
E4-light chain
MluI
LCDR1
LCDR2
LCDR3
NotI
amber-st
6xHis
c-myc
pIII
1 CTCGAGAGCG GGCAGTGAGC GCAACGCAAT TAATGTGAGT TAGCTCACTC ATTAGGCACC CCAGGCTTTA CACTTTATGC
GAGCTCTCGC CCGTCACTCG CGTTGCGTTA ATTACACTCA ATCGAGTGAG TAATCCGTGG GGTCCGAAAT GTGAAATACG
81 TTCCGGCTCG TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTCACACA GAATTCATTA AAGAGGAGAA ATTAACCATG
AAGGCCGAGC ATACAACACA CCTTAACACT CGCCTATTGT TAAAGTGTGT CTTAAGTAAT TTCTCCTCTT TAATTGGTAC
161 AAATACCTAT TGCCTACGGC AGCCGCTGGC TTGCTGCTGC TGGCAGCTCA GCCGGCCATG GCGCAGGTGC AGCTGCAGGA
TTTATGGATA ACGGATGCCG TCGGCGACCG AACGACGACG ACCGTCGAGT CGGCCGGTAC CGCGTCCACG TCGACGTCCT
241 GTCTGGGGGA GGCTTGGTAC AGCCTGGGGG GTCCCTGAGA CTCTCCTGTG CAGCCTCTGG ATTCATGTTT AGCAGGTATG
CAGACCCCCT CCGAACCATG TCGGACCCCC CAGGGACTCT GAGAGGACAC GTCGGAGACC TAAGTACAAA TCGTCCATAC
321 CCATGAGCTG GGTCCGCCAG GCTCCAGGGA AGGGGCCAGA GTGGGTCTCA GGTATTAGTG GTAGTGGTGG TAGTACATAC
GGTACTCGAC CCAGGCGGTC CGAGGTCCCT TCCCCGGTCT CACCCAGAGT CCATAATCAC CATCACCACC ATCATGTATG
401 TACGCAGACT CCGTGAAGGG CCGGTTCACC GTCTCCAGAG ACAATTCCAA GAACACGCTG TATCTGCAAA TGAACAGCCT
ATGCGTCTGA GGCACTTCCC GGCCAAGTGG CAGAGGTCTC TGTTAAGGTT CTTGTGCGAC ATAGACGTTT ACTTGTCGGA
481 GAGAGCCGAG GACACGTCTT CAGCGCTGAG CTCGAAGACT GGTCACCGTC TCCTCAGGGA GTGCATCCGC CCCAAAGCTT
CTCTCGGCTC CTGTGCAGAA GTCGCGACTC GAGCTTCTGA CCAGTGGCAG AGGAGTCCCT CACGTAGGCG GGGTTTCGAA
561 GAAGAAGGTG AATTTTCAGA AGCACGCGTA TCTGAACTGA CTCAGGACCC TGCTGTGTCT GTGGCCTTGG GACAGACAGT
CTTCTTCCAC TTAAAAGTCT TCGTGCGCAT AGACTTGACT GAGTCCTGGG ACGACACAGA CACCGGAACC CTGTCTGTCA
641 CAGGATCACA TGCCAAGGAG ACAGCCTCAG AAACTTTTAT GCAAGCTGGT ACCAGCAGAA GCCAGGACAG GCCCCTACTC
GTCCTAGTGT ACGGTTCCTC TGTCGGAGTC TTTGAAAATA CGTTCGACCA TGGTCGTCTT CGGTCCTGTC CGGGGATGAG
721 TTGTCATCTA TGGTTTAAGT AAAAGGCCCT CAGGGATCCC AGACCGATTC TCTGCCTCCA GCTCAGGAAA CACAGCTTCC
AACAGTAGAT ACCAAATTCA TTTTCCGGGA GTCCCTAGGG TCTGGCTAAG AGACGGAGGT CGAGTCCTTT GTGTCGAAGG
801 TTGACCATCA CTGGGGCTCA GGCGGAAGAT GAGGCTGACT ATTACTGTAA CTCCCGGGAC AGAAGTGGTA ATCATGTAAA
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881 TGTGCTATTC GGCGGAGGGA CCAAGCTGAC CGTCCTACGT CAGCCCAAGG CTGCCCCCTC GGTCACTCTG TTCCCGCCCT
ACACGATAAG CCGCCTCCCT GGTTCGACTG GCAGGATGCA GTCGGGTTCC GACGGGGGAG CCAGTGAGAC AAGGGCGGGA
961 CTTCTGCGGC CGCTGGATCC CATCACCATC ACCATCACTA GGAACAAAAG CTGATCTCAG AAGAAGACCT AAACGGATCC
GAAGACGCCG GCGACCTAGG GTAGTGGTAG TGGTAGTGAT CCTTGTTTTC GACTAGAGTC TTCTTCTGGA TTTGCCTAGG
1041 AAAGATATCA GAGCTGAAAC TGTTGAAAGT TGTTTAGCAA AATCCCATAC AGAAAATTCA TTTACTAACG TCTGGAAAGA
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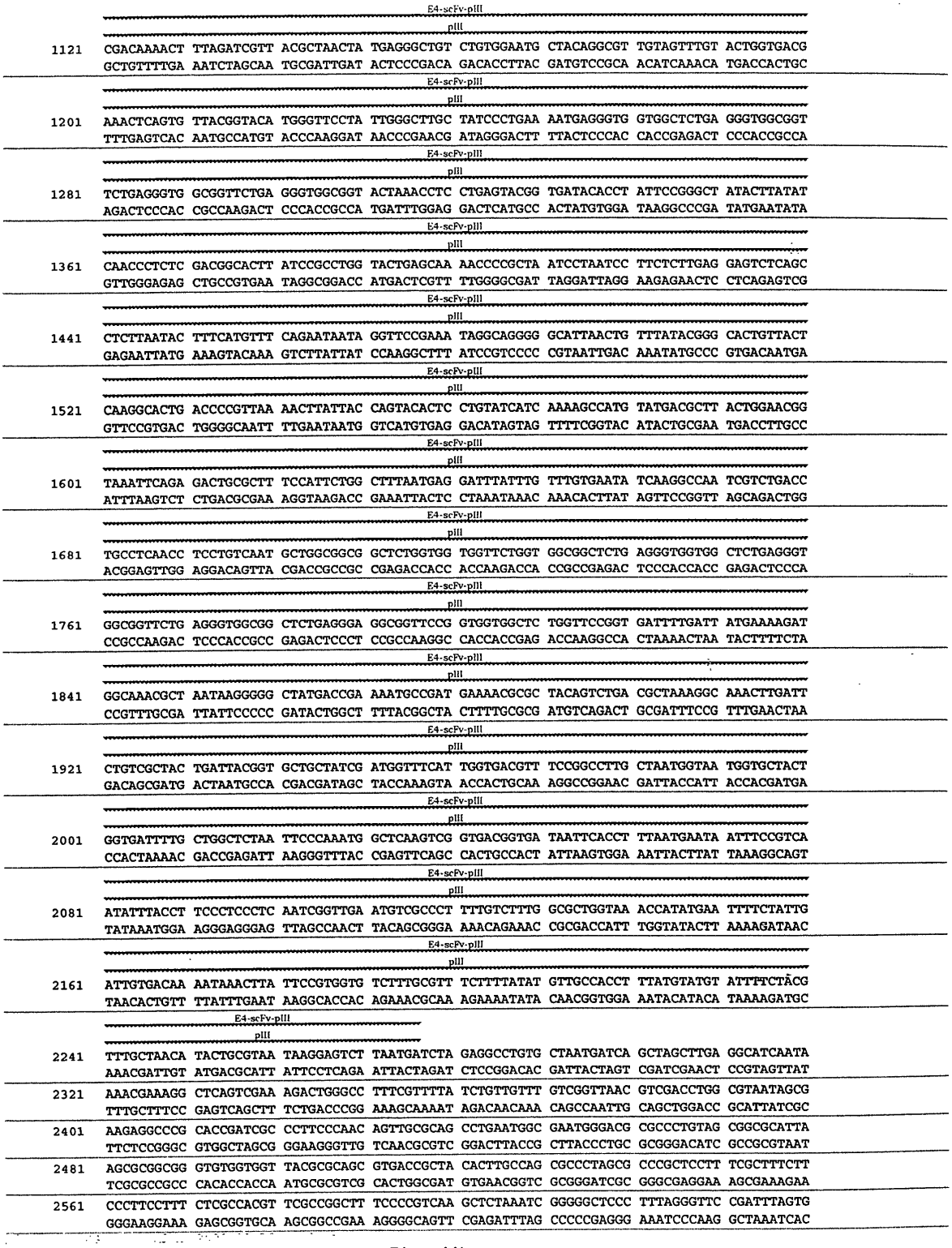
Fig. 11a


E4-scFv-pIII
pIII
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GCTGTTTTGA AATCTAGCAA TGCGATTGAT ACTCCCGACA GACACCTTAC GATGTCCGCA ACATCAAACA TGACCACTGC
1201 AAACTCAGTG TTACGGTACA TGGGTTCCTA TTGGGCTTGC TATCCCTGAA AATGAGGGTG GTGGCTCTGA GGGTGGCGGT
TTTGAGTCAC AATGCCATGT ACCCAAGGAT AACCCGAACG ATAGGGACTT TTACTCCCAC CACCGAGACT CCCACCGCCA
1281 TCTGAGGGTG GCGGTTCTGA GGGTGGCGGT ACTAAACCTC CTGAGTACGG TGATACACCT ATTCCGGGCT ATACTTATAT
AGACTCCCAC CGCCAAGACT CCCACCGCCA TGATTTGGAG GACTCATGCC ACTATGTGGA TAAGGCCCGA TATGAATATA
1361 CAACCCTCTC GACGGCACTT ATCCGCCTGG TACTGAGCAA AACCCCGCTA ATCCTAATCC TTCTCTTGAG GAGTCTCAGC
GTTGGGAGAG CTGCCGTGAA TAGGCGGACC ATGACTCGTT TTGGGGCGAT TAGGATTAGG AAGAGAACTC CTCAGAGTCG
1441 CTCTTAATAC TTTCATGTTT CAGAATAATA GGTTCCGAAA TAGGCAGGGG GCATTAACTG TTTATACGGG CACTGTTACT
GAGAATTATG AAAGTACAAA GTCTTATTAT CCAAGGCTTT ATCCGTCCCC CGTAATTGAC AAATATGCCC GTGACAATGA
1521 CAAGGCACTG ACCCCGTTAA AACTTATTAC CAGTACACTC CTGTATCATC AAAAGCCATG TATGACGCTT ACTGGAACGG
GTTCCGTGAC TGGGGCAATT TTGAATAATG GTCATGTGAG GACATAGTAG TTTTCGGTAC ATACTGCGAA TGACCTTGCC
1601 TAAATTCAGA GACTGCGCTT TCCATTCTGG CTTTAATGAG GATTTATTTG TTTGTGAATA TCAAGGCCAA TCGTCTGACC
ATTTAAGTCT CTGACGCGAA AGGTAAGACC GAAATTACTC CTAAATAAAC AAACACTTAT AGTTCCGGTT AGCAGACTGG
1681 TGCCTCAACC TCCTGTCAAT GCTGGCGGCG GCTCTGGTGG TGGTTCTGGT GGCGGCTCTG AGGGTGGTGG CTCTGAGGGT
ACGGAGTTGG AGGACAGTTA CGACCGCCGC CGAGACCACC ACCAAGACCA CCGCCGAGAC TCCCACCACC GAGACTCCCA
1761 GGCGGTTCTG AGGGTGGCGG CTCTGAGGGA GGCGGTTCCG GTGGTGGCTC TGGTTCCGGT GATTTTGATT ATGAAAAGAT
CCGCCAAGAC TCCCACCGCC GAGACTCCCT CCGCCAAGGC CACCACCGAG ACCAAGGCCA CTAAAACTAA TACTTTTCTA
1841 GGCAAACGCT AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCGC TACAGTCTGA CGCTAAAGGC AAACTTGATT
CCGTTTGCGA TTATTCCCCC GATACTGGCT TTTACGGCTA CTTTTGCGCG ATGTCAGACT GCGATTTCCG TTTGAACTAA
1921 CTGTCGCTAC TGATTACGGT GCTGCTATCG ATGGTTTCAT TGGTGACGTT TCCGGCCTTG CTAATGGTAA TGGTGCTACT
GACAGCGATG ACTAATGCCA CGACGATAGC TACCAAAGTA ACCACTGCAA AGGCCGGAAC GATTACCATT ACCACGATGA
2001 GGTGATTTTG CTGGCTCTAA TTCCCAAATG GCTCAAGTCG GTGACGGTGA TAATTCACCT TTAATGAATA ATTTCCGTCA
CCACTAAAAC GACCGAGATT AAGGGTTTAC CGAGTTCAGC CACTGCCACT ATTAAGTGGA AATTACTTAT TAAAGGCAGT
2081 ATATTTACCT TCCCTCCCTC AATCGGTTGA ATGTCGCCCT TTTGTCTTTG GCGCTGGTAA ACCATATGAA TTTTCTATTG
TATAAATGGA AGGGAGGGAG TTAGCCAACT TACAGCGGGA AAACAGAAAC CGCGACCATT TGGTATACTT AAAAGATAAC
2161 ATTGTGACAA AATAAACTTA TTCCGTGGTG TCTTTGCGTT TCTTTTATAT GTTGCCACCT TTATGTATGT ATTTTCTACG
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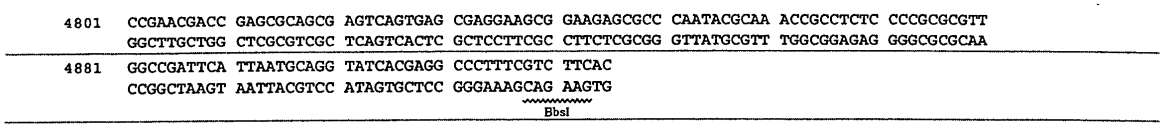
2241 TTTGCTAACA TACTGCGTAA TAAGGAGTCT TAATGATCTA GAGGCCTGTG CTAATGATCA GCTAGCTTGA GGCATCAATA
AAACGATTGT ATGACGCATT ATTCCTCAGA ATTACTAGAT CTCCGGACAC GATTACTAGT CGATCGAACT CCGTAGTTAT
2321 AAACGAAAGG CTCAGTCGAA AGACTGGGCC TTTCGTTTTA TCTGTTGTTT GTCGGTTAAC GTCGACCTGG CGTAATAGCG
TTTGCTTTCC GAGTCAGCTT TCTGACCCGG 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

Fig. 11b

```
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
                                                  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
```

Fig. 11c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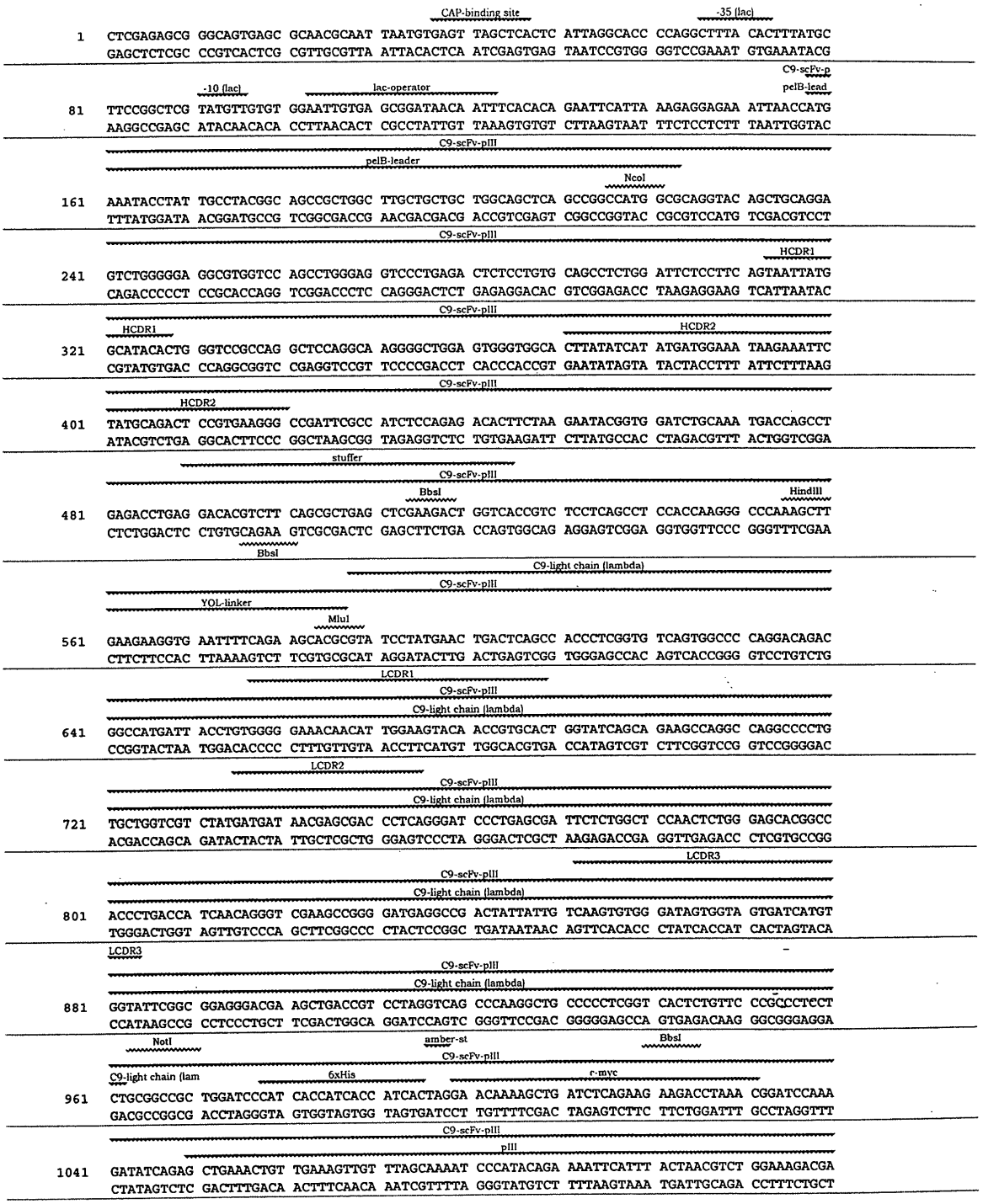
BbsI

Fig. 11d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C9-scFv-p
-10 (lac)
lac-operator
pelB-lead
81 TTCCGGCTCG TATGTTGTGT GGAATTGTGA GCGGATAACA ATTTCACACA GAATTCATTA AAGAGGAGAA ATTAACCATG
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C9-scFv-pIII
pelB-leader
NcoI
161 AAATACCTAT TGCCTACGGC AGCCGCTGGC TTGCTGCTGC TGGCAGCTCA GCCGGCCATG GCGCAGGTAC AGCTGCAGGA
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HCDR1
HCDR2
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401 TATGCAGACT CCGTGAAGGG CCGATTCGCC ATCTCCAGAG ACACTTCTAA GAATACGGTG GATCTGCAAA TGACCAGCCT
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stuffer
C9-scFv-pIII
BbsI
HindIII
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BbsI
C9-light chain (lambda)
C9-scFv-pIII
YOL-linker
MluI
561 GAAGAAGGTG AATTTTCAGA AGCACGCGTA TCCTATGAAC TGACTCAGCC ACCCTCGGTG TCAGTGGCCC CAGGACAGAC
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LCDR1
C9-scFv-pIII
C9-light chain (lambda)
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C9-light chain (lambda)
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LCDR3
C9-scFv-pIII
C9-light chain (lambda)
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LCDR3
C9-scFv-pIII
C9-light chain (lambda)
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NotI
amber-st
BbsI
C9-scFv-pIII
C9-light chain (lam
6xHis
c-myc
961 CTGCGGCCGC TGGATCCCAT CACCATCACC ATCACTAGGA ACAAAAGCTG ATCTCAGAAG AAGACCTAAA CGGATCCAAA
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C9-scFv-pIII
pIII
1041 GATATCAGAG CTGAAACTGT TGAAAGTTGT TTAGCAAAAT CCCATACAGA AAATTCATTT ACTAACGTCT GGAAAGACGA
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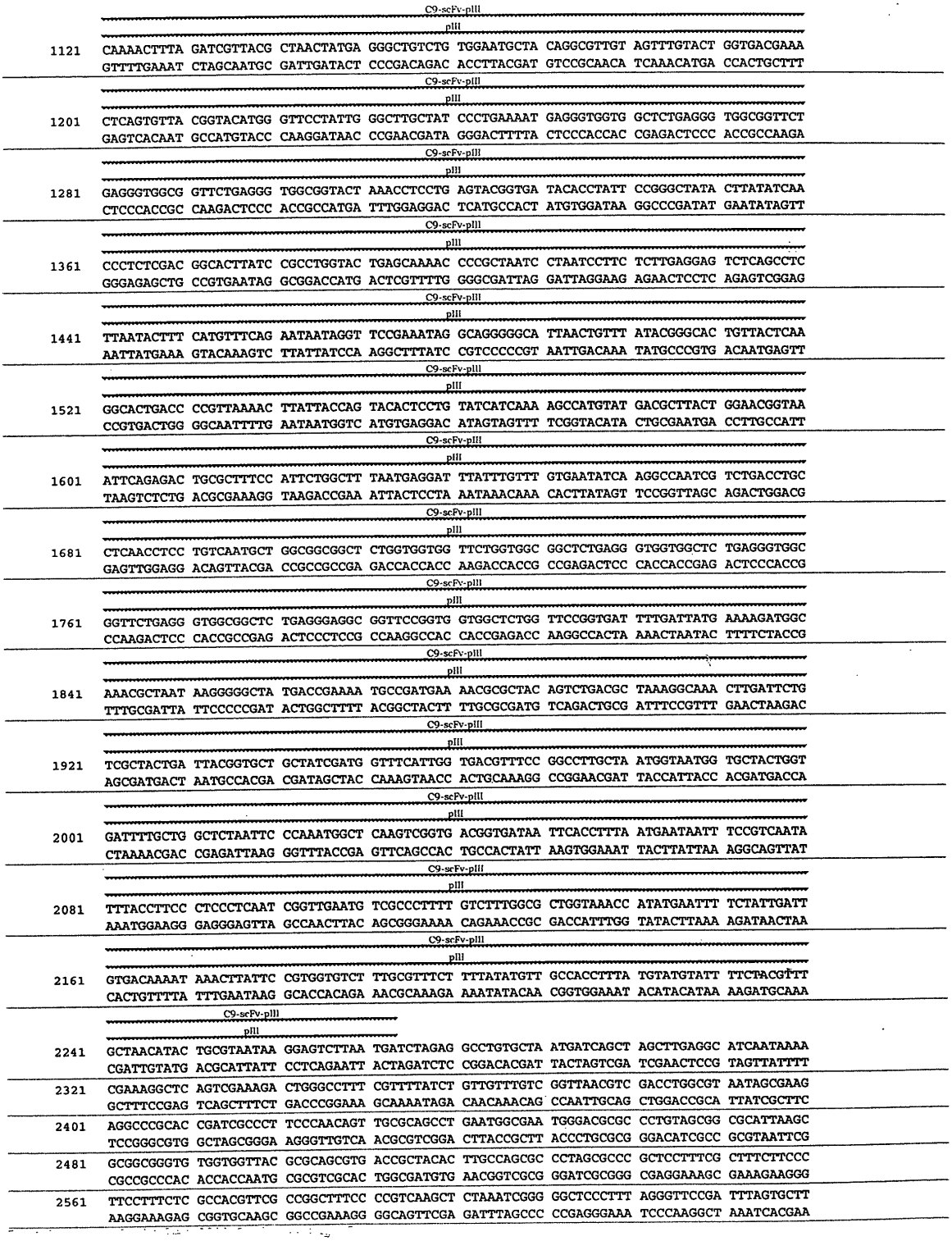
Fig. 12a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1201 CTCAGTGTTA CGGTACATGG GTTCCTATTG GGCTTGCTAT CCCTGAAAAT GAGGGTGGTG GCTCTGAGGG TGGCGGTTCT
GAGTCACAAT GCCATGTACC CAAGGATAAC CCGAACGATA GGGACTTTTA CTCCCACCAC CGAGACTCCC ACCGCCAAGA
1281 GAGGGTGGCG GTTCTGAGGG TGGCGGTACT AAACCTCCTG AGTACGGTGA TACACCTATT CCGGGCTATA CTTATATCAA
CTCCCACCGC CAAGACTCCC ACCGCCATGA TTTGGAGGAC TCATGCCACT ATGTGGATAA GGCCCGATAT GAATATAGTT
1361 CCCTCTCGAC GGCACTTATC CGCCTGGTAC TGAGCAAAAC CCCGCTAATC CTAATCCTTC TCTTGAGGAG TCTCAGCCTC
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1441 TTAATACTTT CATGTTTCAG AATAATAGGT TCCGAAATAG GCAGGGGGCA TTAACTGTTT ATACGGGCAC TGTTACTCAA
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1761 GGTTCTGAGG GTGGCGGCTC TGAGGGAGGC GGTTCCGGTG GTGGCTCTGG TTCCGGTGAT TTTGATTATG AAAAGATGGC
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2001 GATTTTGCTG GCTCTAATTC CCAAATGGCT CAAGTCGGTG ACGGTGATAA TTCACCTTTA ATGAATAATT TCCGTCAATA
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C9-scFv-pIII
pIII
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2481 GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC
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2561 TTCCTTTCTC GCCACGTTCG CCGGCTTTCC CCGTCAAGCT CTAAATCGGG GGCTCCCTTT AGGGTTCCGA TTTAGTGCTT
AAGGAAAGAG CGGTGCAAGC GGCCGAAAGG GGCAGTTCGA GATTTAGCCC CCGAGGGAAA TCCCAAGGCT AAATCACGAA

Fig. 12b

```
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
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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
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                                          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
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                                bla
3841  TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT
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3921  CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG
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4001  ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC
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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
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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
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Fig. 12c

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

4881  CGATTCATTA ATGCAGGTAT CACGAGGCCC TTTCGTCTTC AC
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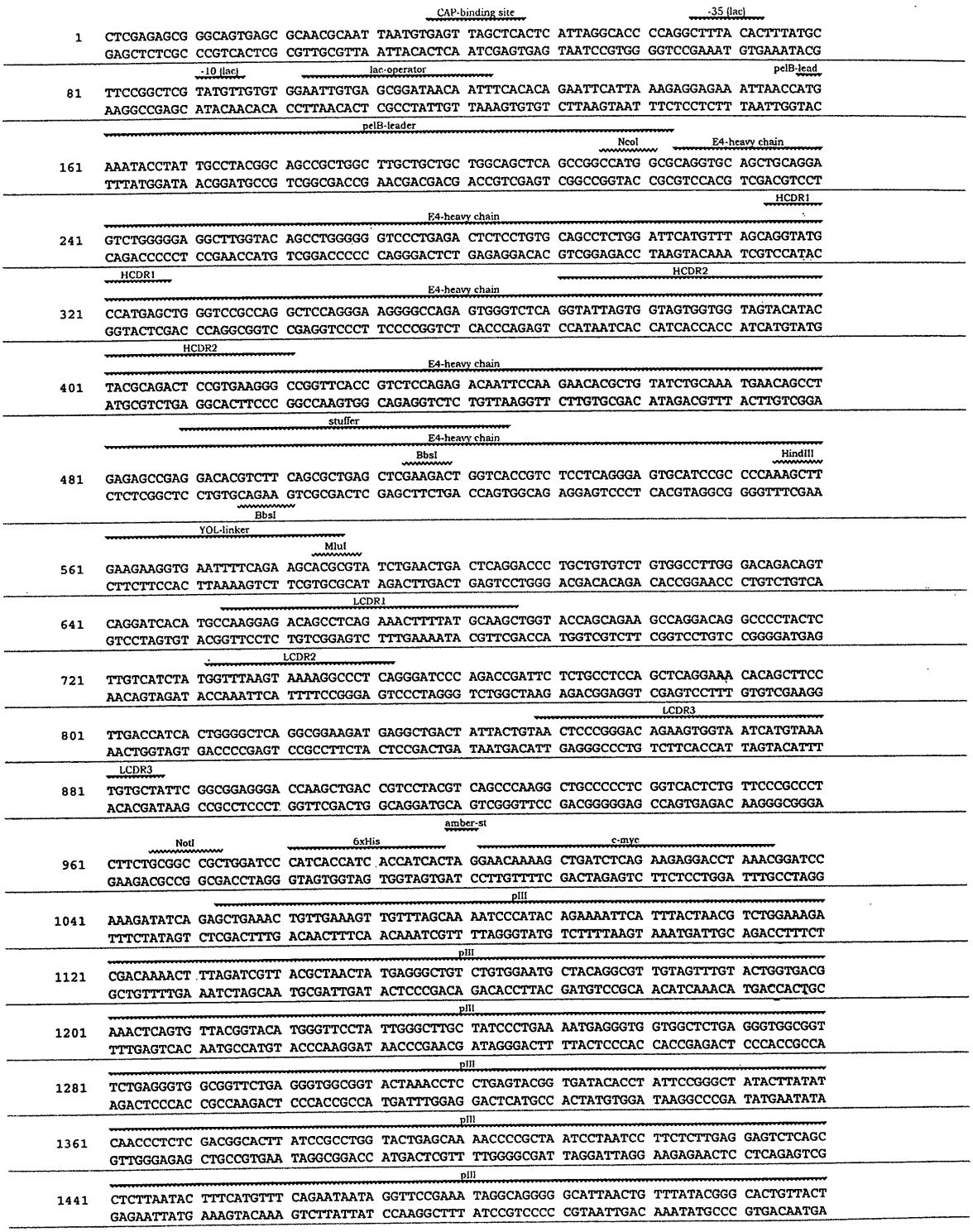
Fig. 12_d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BbsI
HindIII
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amber-st
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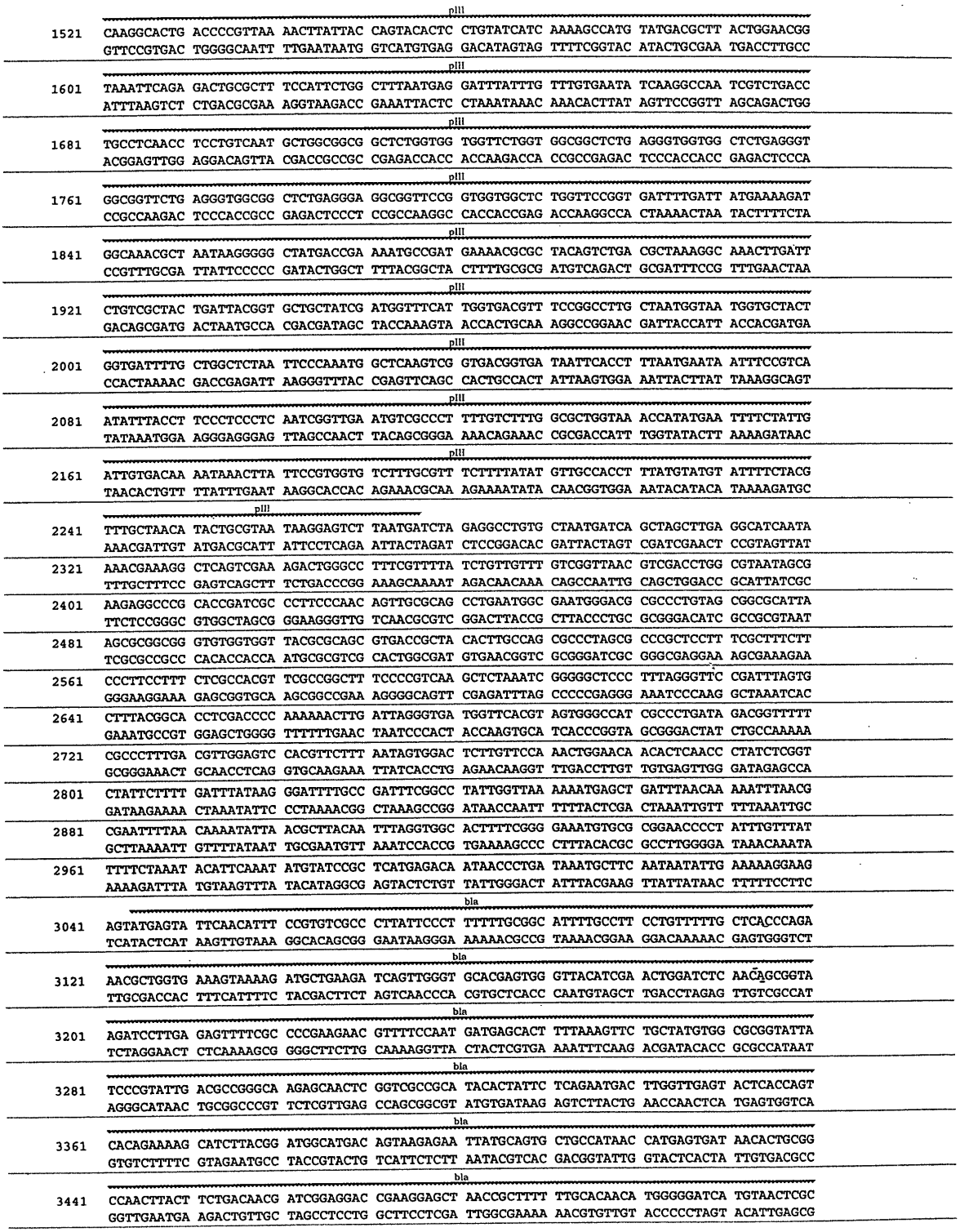
6xHis
c-myc
pIII

Fig. 13a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pIII
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pIII
1681 TGCCTCAACC TCCTGTCAAT GCTGGCGGCG GCTCTGGTGG TGGTTCTGGT GGCGGCTCTG AGGGTGGTGG CTCTGAGGGT
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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
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pIII
2001 GGTGATTTTG CTGGCTCTAA TTCCCAAATG GCTCAAGTCG GTGACGGTGA TAATTCACCT TTAATGAATA ATTTCCGTCA
CCACTAAAAC GACCGAGATT AAGGGTTTAC CGAGTTCAGC CACTGCCACT ATTAAGTGGA AATTACTTAT TAAAGGCAGT
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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
GCTTAAAAATT 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

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
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4161 AGCTACCAAC TCTTTTTCCG AAGGTAACTG GCTTCAGCAG AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG
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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
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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

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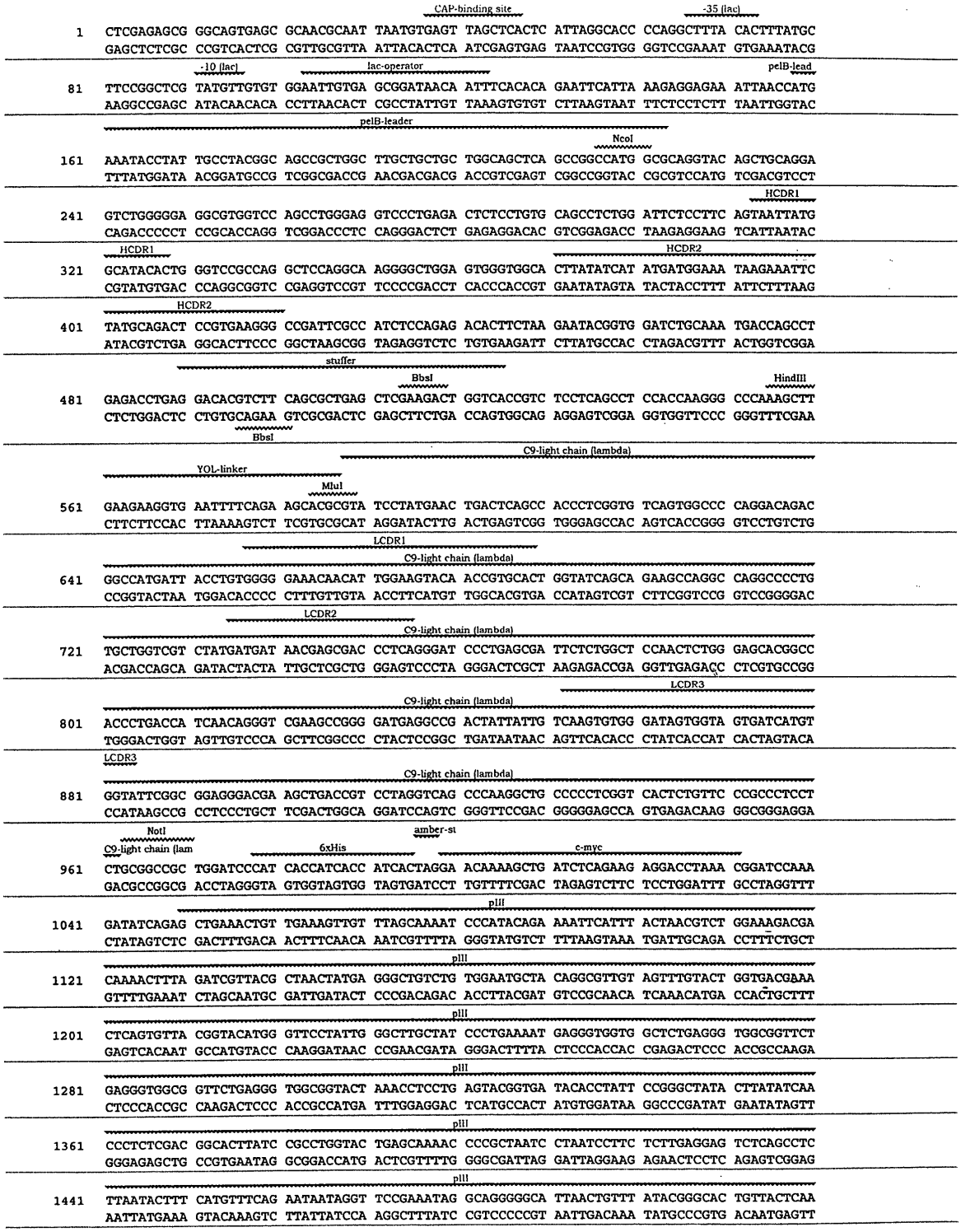
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-10 (lac)
lac-operator
pelB-lead
pelB-leader
NcoI
HCDR1
HCDR1
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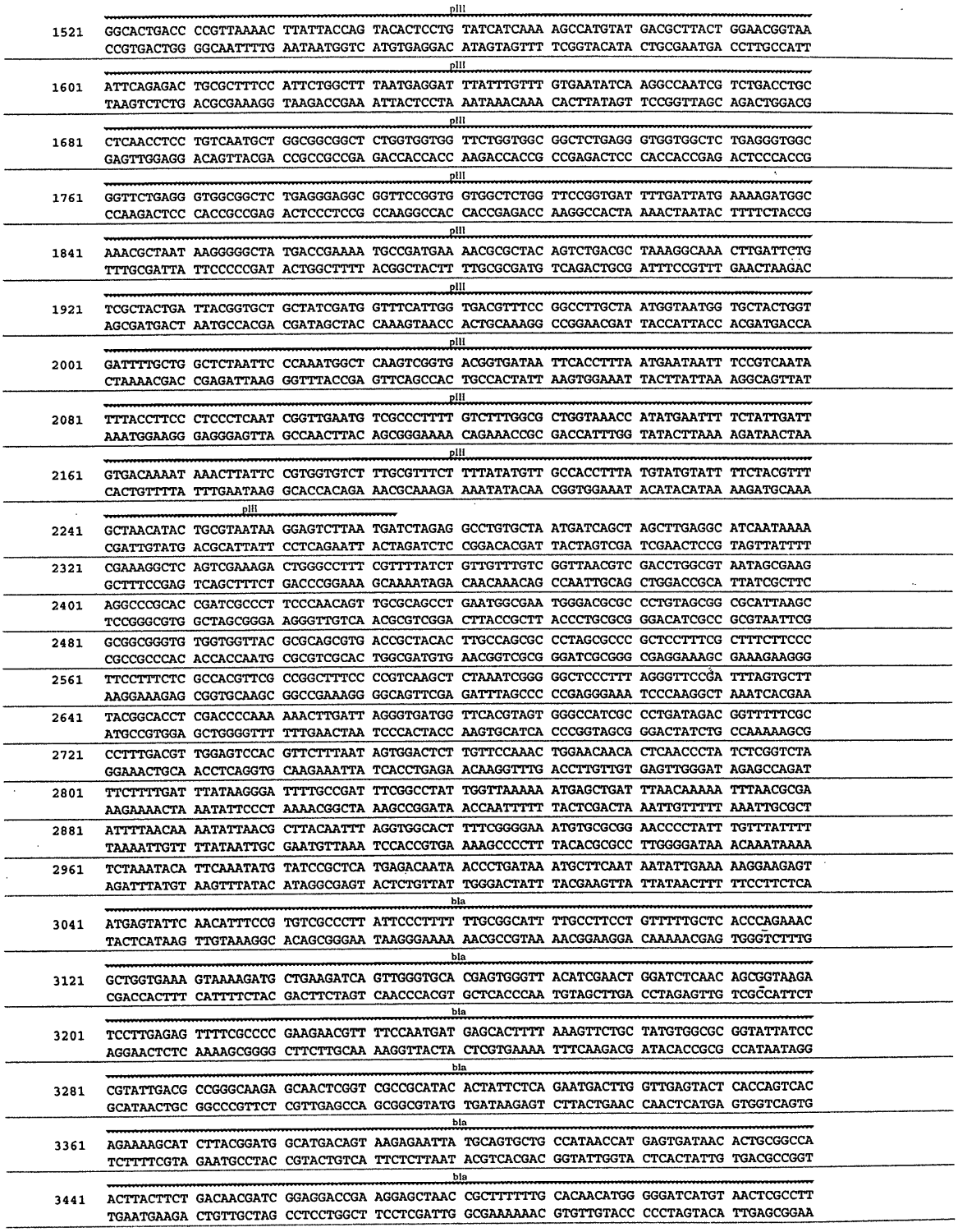
HCDR2
stuffer
BbsI
HindIII
BbsI
C9-light chain (lambda)
YOL-linker
MluI
LCDR1
C9-light chain (lambda)
LCDR2
C9-light chain (lambda)
LCDR3
C9-light chain (lambda)
LCDR3
C9-light chain (lambda)
NotI
C9-light chain (lam
amber-st
6xHis
c-myc
pIII
pIII
pIII
pIII
pIII

Fig. 14a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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
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1761 GGTTCTGAGG GTGGCGGCTC TGAGGGAGGC GGTTCCGGTG GTGGCTCTGG TTCCGGTGAT TTTGATTATG AAAAGATGGC
CCAAGACTCC CACCGCCGAG ACTCCCTCCG CCAAGGCCAC CACCGAGACC AAGGCCACTA AAACTAATAC TTTTCTACCG
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1841 AAACGCTAAT AAGGGGGCTA TGACCGAAAA TGCCGATGAA AACGCGCTAC AGTCTGACGC TAAAGGCAAA CTTGATTCTG
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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
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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
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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
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bla
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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

```
                                                    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
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      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

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 01 12 3851

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	NGUYEN-HO P ET AL: "Platelet glycoprotein IIb / IIIa receptor antagonists and coronary artery disease." CURR ATHEROSCLER REP, (2001 MAR) 3 (2) 139-48. REF: 56 , XP001057096 * the whole document *	1-7, 11-17	C07K16/28 A61K39/395 G01N33/53 C12N15/13 C12N5/10 C12N15/63
A	BERKOWITZ S D: "Current knowledge of the platelet glycoprotein IIb / IIIa receptor antagonists for the treatment of coronary artery disease." HAEMOSTASIS, (2000) 30 SUPPL 3 27-43. REF: 63 , XP001057638 * the whole document *	1-7, 11-17	
A	DOBESH P P ET AL: "Advancing the battle against acute ischemic syndromes: a focus on the GP IIb-IIIa inhibitors." PHARMACOTHERAPY, (1998 JUL-AUG) 18 (4) 663-85. REF: 91 , XP001057099 * the whole document *	1-7, 11-17	
	-/--		

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C07K
A61K
G01N
C12N

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	5 March 2002	Mennessier, T

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 01 12 3851

Claim(s) searched completely:
1-7,11-17

Claim(s) not searched:
8-10

Reason for the limitation of the search:

Insofar as processes for identifying and/or isolating antibodies for inhibiting platelet aggregation by binding to an activated integrin receptor of blood thrombocytes are concerned, the true contribution of the present invention to the art, as explained on description page 14 (see the paragraph entitled "General strategy"), appears to rely on the generation of phage libraries either from IgM antibodies genes or by randomisation of the CDR3 region of the human chain in two scFv master frameworks of human origin. The first aspect appears not to be covered by either claim 8 or dependent claims 9-10. The second aspect is not referred to in any of claims 8-10 but is the subject-matter of claim 11, which is dependent on claim 10. Therefore, claims 8-10 appear to be directed to a non-permitted generalisation of the invention in contradiction with the requirements of Article 84 EPC, the defect being such that a meaningful search could not be carried out on claims 8-10.

In contrast, claim 11 could be searched completely.

Also claims 1-7 and 12-17 which are directed to other aspects of the invention could be searched completely.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 01 12 3851

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	JUBELIRER S J ET AL: "Acute profound thrombocytopenia following C7E3 Fab (Abciximab) therapy: case reports, review of the literature and implications for therapy." AMERICAN JOURNAL OF HEMATOLOGY, (1999 JUL) 61 (3) 205-8. REF: 11 , XP001057619 * the whole document *	1-7, 11-17	
A	SUZUKI K ET AL: "Comparison of the antiplatelet effect of YM337 and abciximab in rhesus monkeys." EUROPEAN JOURNAL OF PHARMACOLOGY, (1997 OCT 8) 336 (2-3) 169-76. , XP001057621 * the whole document *	1-7, 11-17	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

EPO FORM 1503 03.82 (P04C10)

专利名称(译)	用于抑制血小板聚集的人源抗体		
公开(公告)号	EP1300419A1	公开(公告)日	2003-04-09
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	C12N15/09 A61K31/7088 A61K39/395 A61K48/00 A61P7/02 A61P37/02 C07K16/28 C12N1/15 C12N1/19 C12N1/21 C12N5/10 C12N15/13 C12P21/08 C12N15/63 G01N33/53		
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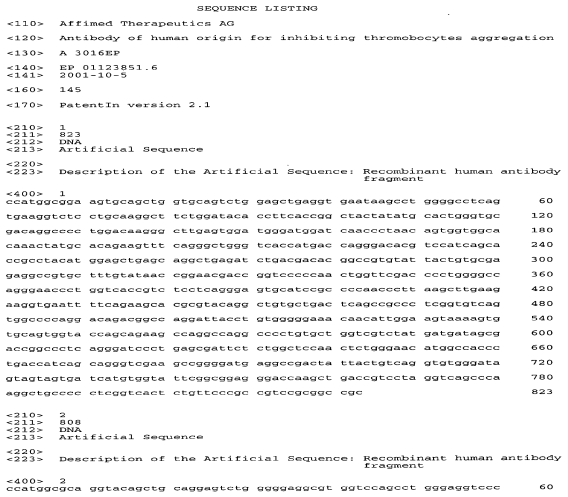
其他公开文献	EP1300419B1		
外部链接	Espacenet		

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。



SEQUENCE LISTING

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<120> Antibody of human origin for inhibiting thromobocytes aggregation

<130> A 3016EP

<140> EP 01123851.6
<141> 2001-10-5

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