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(54) Title: DUAL CISTRONIC BACTERIAL EXPRESSION SYSTEM

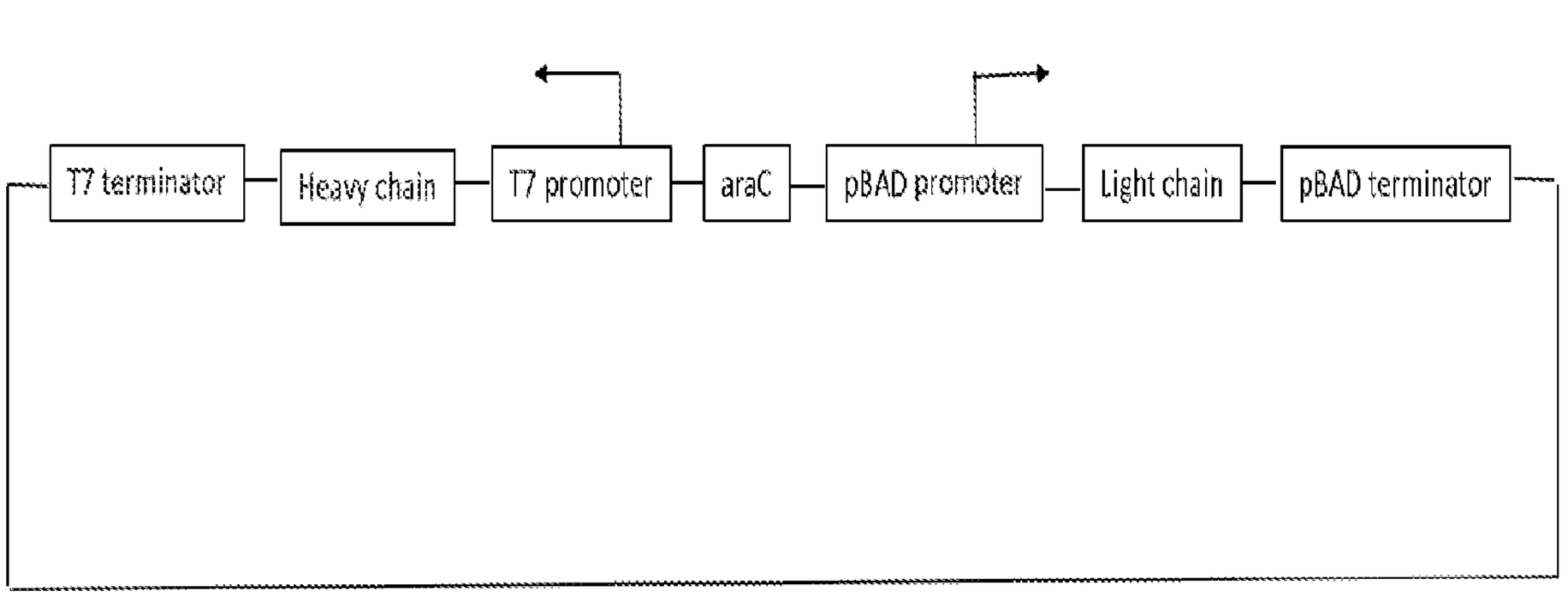

Figure 1

(57) Abrégé/Abstract:
The present invention relates to the dual, independent cistron expression system in a single vector for the production of protein of interest proteins and peptides expressed as insoluble inclusion bodies formed in the bacteria E. coli.The present invention also provides the process for the expression of protein of interest using said bicistronic vector.

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(54) **Title**: DUAL CISTRONIC BACTERIAL EXPRESSION SYSTEM

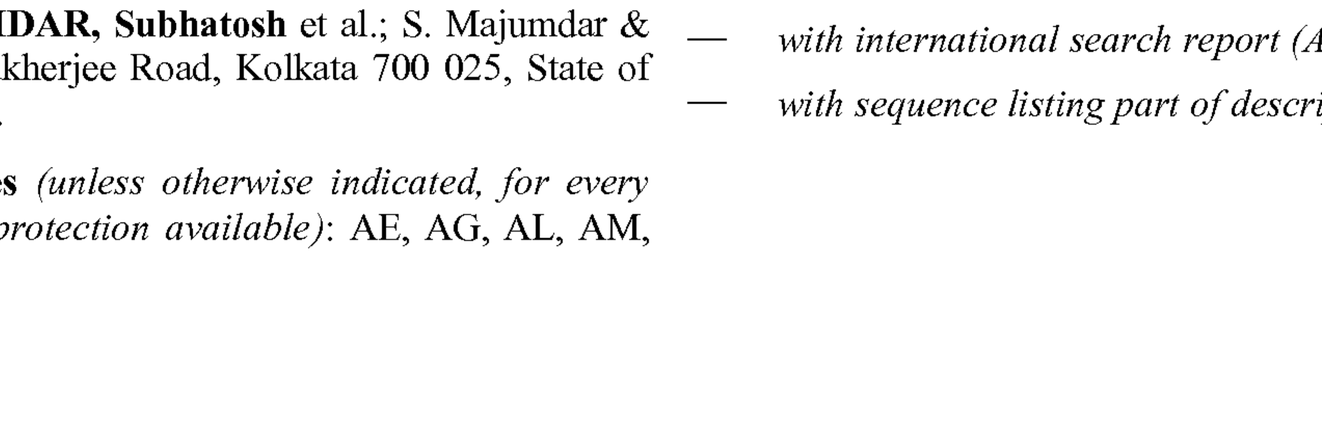

Figure 1

(57) **Abstract**: The present invention relates to the dual, independent cistron expression system in a single vector for the production of protein of interest proteins and peptides expressed as insoluble inclusion bodies formed in the bacteria *E. coli*.The present invention also provides the process for the expression of protein of interest using said bicistronic vector.

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DUAL CISTRONIC BACTERIAL EXPRESSION SYSTEM

Field Of The Invention

The present invention relates to the dual, independent cistron expression system in a single vector for the production of protein of interest comprises recombinant Fab fragments of antibodies or other antibody fragment, peptides and proteinsexpressedas insoluble inclusion bodies formed in the bacteria *E. coli*.The present invention also provides the process for the expression of protein of interest using said bicistronic vector.

Background Of The Invention

Recombinant DNA technology (rDNA) has revolutionized the way therapeutic agents are prepared. The required proteins are now made inside a foreign cell and purified.

Proteins having post-translational modifications (PTMs) are generally expressed as recombinant molecules in mammalian or yeast system. The yeast expression systems like Pichia and Saccharomyces are closer to mammalian systems in terms of PTMs but still differ in the types of glycosylations like high mannose glycans in case of Pichia make them unsuitable for expression of recombinant proteins for human use.

Monoclonal antibodies (mAbs), antibodies, fusion proteins, Fab fragments of mAbs are used as therapeutic agents. The rDNA technology uses specialized vectors and expression systems for production of therapeutic proteins. The expression systems mainly consist of bacterial, yeast, insect or mammalian expression systems. Initially, most of the recombinant proteins were expressed in bacterial expression system using *E. coli* as host. There are several advantages of using*E. coli* as expression host such as ease of cloning, ease of expression, shorter timelines, shorter incubation periods and very high yields. Thus, proteins which do not need any PTMs can be safely expressed in *E. coli.*

Fabs, which are antigen binding fragment part of mAbs, need not to be expressed in mammalian systems as they do not contain glycosylation sites present in the Fc portion of the antibody. Hence, Fabs are usually expressed in *E. coli* system. During 1980-90s several

researchers attempted the expression of Fabs in *E. coli*. PlückthunA*et. al.*,1990*Behring Inst. Mitt.* (87):48-55are some of the earlier workers who reported secretion of Fab antibody from *E. coli*. Williamson R.A.*et. al.*, 1991 *Biochem J.* 277 (Pt 2):561-3 reported use of bacteriophage lambda vectors for expression of Fab molecules in*E. coli*. Phage display system for production of Fab, bivalent antibody or chimeric antibody fragments in *E. coli*. Moreover, Fab was also produced in *E. coli* as misfolded, inclusion bodies and then refolded them to get the functional molecule and thereby 40% increase in the yields of antibody was obtained.

Most of the studies mentioned above used single promoter, i.e., phoA to drive the expression of both heavy and light chains. The ribosome binding site (rbs) present in between heavy and light chains drives the transcription and translation of second gene.

US5648237 also used similar single promoter (phoA) strategy to express Fabgenes in *E. coli* to get secreted product. The major drawback of the above strategy is that the expression levels of the second gene are usually lower than first gene, thus limiting the yields of the functional Fab.

Patent No. WO03018771 discloses a process for producing an antibody by two separate translational units, respectively encoding the light and heavy chains of said antibody or fragment, wherein both the chains are expressed in a sequential fashion, thereby specifically separating the production of the light and heavy chains and allowing the assembly of the light and heavy chains.

Patent No. EP1356052B1 discloses a method to produce full antibodies in prokaryotic cells. There is a presence of a first promoter and a first cistron to produce immunoglobulin light chain and a second promoter and a second cistron to produce immunoglobulin heavy chain, wherein both the chains are folded and assembled to form a biologically active immunoglobulin.

Summary Of The Invention

In an embodiment, the invention is related to a dual, independent cistron expression system in a single vector for the production of recombinant proteins and peptides expressed as insoluble inclusion bodies in bacterial cells.

In another embodiment, the invention is related to aprocess of preparation of dual, independent cistron expression system in a single vector having two different promoters for production of recombinant proteins and peptides expressed as insoluble inclusion bodies in bacterial cells.

In another embodiment, the invention is related to a dual, independent cistron expression system in a single vector having two different promoters for production of antibody fragments expressed as insoluble inclusion bodies in the bacterial cells.

In another embodiment, the invention is related to a dual, independent cistron expression system in a single vector having two different promoters for production of recombinant Fab fragment of antibodies expressed as insoluble inclusion bodies in the bacterial cells.

In another embodiment, the invention is related to a dual, independent cistron expression system in a single vector having two different promoters for production of recombinant peptides expressed as insoluble inclusion bodies in the bacterial cells.

In another embodiment the dual cistronic expression system comprises:

a) firstcistron comprising a promoter operably linked with polynucleotide sequence encoding protein of interest;

b) secondcistron comprising a promoter operably linked with polynucleotide sequence encoding protein of interest;

wherein the first and second cistrons are positioned in single vector and express a polynucleotide sequence encoding the protein of interest as inclusion bodies in bacterial cell.

In another embodiment the invention relates to dual cistronic vector comprising promoter operably linked to multiple cloning site contain gene of interest, ribosome binding site and terminator.

In yet another embodiment the invention relates to process for producing a protein of interest using dual cistronic expression system.

The details of one or more embodiments of the invention set forth below are illustrative in nature only and not intended to limit the scope of the invention. Other features, objects and advantages of the inventions will be apparent from the description.

Brief description of accompanying figures

Figure 1; illustrates the formula of bicistronic vector

Figure 2; illustrates the vector map of clone pET21a-HC-LC

Figure 3; illustrates the SDS PAGE analysis of insoluble pellet fraction of *E. coli* BL21A1 clone along with controls and the reference product

Figure 4; illustrates the RP-HPLC analysis of solubilized IB samples with LC and HC peaks seen in clone compared to reduced Fab molecule

Figure 5; illustrates the HPLC runs to seperate heavy chain peak from other proteins

Figure 6; illustrates significant increase in expression of SAK-Lira clone in dual cistron construct as compared with single cistron clone in *E. coli*BL21 A1 cell line.

Figure 7; illustrates the vector map pBAD24M-LC

Detailed Description of the Invention

Definations:

As used herein, the term, “Protein of interest” refers herein to any polypeptide including protein and peptides used in biotherapeutic industry or for diagnostic or research purpose.

As used herein, the term "polynucleotide sequence encoding a protein of interest" as used herein includes DNA coding for a gene, preferably a heterologous gene expressing the polypeptide.

As used herein, the terms "recombinant protein and peptide" refers to a protein or peptide that is produced by the expression of recombinant DNA within living cells.

As used herein, the terms "Fab" and "antibody" are used interchangeably because antibody comprises two parts, i.e., Fab and Fc region.

As used herein, the term “vector” refers to a DNA molecule used as a vehicle to artificially carry foreign genetic material into bacterial cell, where it can be replicated and expressed.

As used herein, the term “cistron” refers to a section of DNA that contains the genetic code for a single polypeptide and functions as a hereditary unit.

As used herein, the term "dual independent cistron expression" refers to two separate cistrons which are used to express two same or different proteins independently.

As used herein, the term “same” is interchanble with identical or similar.

The term "Dual cistronic expression system" as used herein includes a polynucleotide sequence encoding a polypeptide to be expressed and sequences controlling its expression such as a promoter and optionally an enhancer sequence. The promoter of the invention is either operably linked to the gene to be expressed, i.e. transcription unit, or is separated therefrom by intervening DNA such as for example by the 5 '-untranslated region of the

heterologous gene. Preferably the expression system is flanked by one or more suitable restriction sites in order to enable the insertion of the expression cassette into a vector and/or its excision from a vector. Thus, the expression system according to the present invention may be used for the construction of an expression vector, in particular a bacterial expression vector.

As used herein, the term “promoters” refers to a regulatory region of DNA usually located upstream of a gene, providing a control point for regulated gene transcription.

As used herein, the terms "operably linked" refer to a functional relationship between two or more DNA segments, in particular gene sequences to be expressed and those sequences controlling their expression.

As used herein, the term “small peptides” or “peptides” refers to peptides ranging from 2 to 10 kDa used in biotherapeutic industry and diagnostic and research purposes like Liraglutide, exanetide, PTH, etc..

The present invention provides a dual cistronic expression system for the production of variety of recombinant protein of interest. In certain embodiment the dual cistronic expression system comprises two cistrons having promoter operably linked with polynucleotide sequence encoding protein of interest and terminator.

In certain embodiment the dual cistronic expression system comprises two cistrons express polynucleotide sequence encoding protein of interest are positioned in single vector.

In an embodiment the dual cistronic expression system comprises;

a)firstcistron comprising a promoter operably linked with polynucleotide sequence encoding protein of interest;

b)secondcistron comprising a promoter operably linked with polynucleotide sequence encoding protein of interest;

wherein the first and second cistrons are positioned in single vector and express a polynucleotide sequence encoding the protein of interest as inclusion bodies formed in host cell.

In an embodiment the promoter may be selected from T7 promoter, arabinose promoter phoA, tac, lpp, lac-lpp, lac, trp, trc, preferably T7 promoter and arabinose promoter.In certain embodiment the dual cistronic expression system comprises two cistrons express polynucleotide sequence encoding protein of interest under the control of two promoters.In one embodiment both promoters control the expression of polynucleotide sequence encoding the same protein of interest.In another embodiment both promoters control the expression of polynucleotide sequence encoding protein of interest different in length of amino acid or physio-chemical properties.

In certain embodiment the protein of interest may be selected frompeptides and proteins.

In some embodiment the proteins may be expressed in bicistronic vector. The protein comprises antibody or fragment thereof. Antibody fragment may be expressed in bicistronicexpression system. The antibody fragment may be selected from Fab heavy chain and light chains of antibodies or other antibody fragments such as scFv, Diabodies, Triabodies, Tetrabodies, Bis-scFv, Minibodies Fab_2 (bispecific), Fab3 (trispecific). In preferred embodiment the bicistronic expression system express polynucleotide sequence encoding heavy chain and light chain of antibody which forms a Fab antibody. In such embodiment the Fab antibody shows affinity to VEGF receptor and said Fab antibody is Ranibizumab.

In another embodiment the protein may be selected from but not limiting to G-CSF, IFN, erythropoietin, insulin and its varients, PTH (1-84aa), FSH, LH, GH and Protein disulfide isomerase (PDI).

In some embodiment the peptidesmay be expressed in bicistronic vector. The peptidescomprise amino acid sequence are selected from at least less than 40 amnio acid or preferably less than 31 amino acid or more preferably less than 10 amino acid.In certain embodiment the peptide molecular weight is selected from about 2 to about 10 kDa. The peptide maybe selected from but not limiting to GLP-1 peptide analoguessuch as Liraglutide or Exendinor GLP-2 peptide like teduglutideand PTH (1-34aa) and insulin. In another preferred embodiment the bicistronic expression system express polynucleotide sequence encoding GLP-1 agonist peptide. In such embodiment the GLP-1 peptide is Liraglutide.

In another embodiment both promoters independently control the expression of different protein of interest such as heavy chain or light chain of antibody which are different in length of amino acid and physio-chemical property.

In an embodiment the dual cistronic expression system comprises:

a) first cistron comprises T7 promoter operably linked with polynucleotide sequence encoding heavy chain of antibody;

b) secondcistron comprises arabinose promoter operably linked with polynucleotide sequence encoding light chain of antibody;

Wherein the first and second cistrons are positioned in single vector and express the heavy chain and light chain of the antibody as inclusion bodies formed in host cell.

In such embodiment the antibody heavy chain and light chain of antibody comprise the nucleotide sequence sequence ID nos. 1and sequence ID nos. 2 or amino acid sequence sequence ID nos. 3 and sequence ID nos.4.In some embodiment the position of first and second cistron is interchangeable wherein the second cistronmay be cloned in vector at the

position of first cistron and first cistronmay be positioned at second cistron. The heavy chain and light chain of antibody independently express as inclusion bodies and may be further treated to obtain Fab antibody which shows affinity to VEGF receptor and said Fab antibody is Ranibizumab.

In certain embodiment the heavy chain and light chain of antibody are optionally expressed in combination with signal peptide, preferably pelB. Signal peptide directs the expression of protein in periplasmic space of the host cell.

In embodiment, the dualcistronicexpression system in a single vector having two different promoters, arabinose and T7 promoters regulating the production of heavy and light chains of recombinant Fab fragments, respectively and both having a pelB tag produced as insoluble inclusion bodies in theperiplasmic space of *E. coli*.

In certain embodiment the heavy chain or light chain of antibody is optionally expressed in combination with regulator, preferably AraC gene in order to further increase the expression of protein.

The dual cistronic expression system provides equimolar expression of protein of interest. Equimolar expression is highly desirable in order to obtain protein of interest in suitable quality and quantity. It depends over the ratio of heavy and light chain or the ratio of subunit of polypeptide cloned in to the vector. In certain embodiment the the heavy chain and light chain are cloned in suitable ratio comprises the heavy chain is at least equal or higher than light chain to obtain equimolar expression of heavy and light chain. The heavy chain and light chain are cloned in a ratio selected from 1:5:0.7 to 1:1 which includes 1:3:0.8, 1:2:0.9, 1:2:1 1:1.

In embodiment the dual cistronic expression system comprises nucleotide sequence as set forth in sequence ID nos 19.

In another embodiment the dual cistronic expression system comprising:

a) firstcistron comprises T7 promoter operably linked with polynucleotide sequence encoding peptide;

b) secondcistron comprises arabinose promoter operably linked with polynucleotide sequence encoding peptide;

wherein the first and second cistrons are positioned in single vector and express the peptide as inclusion bodies formed in host cell.

In such embodiment, the peptide is a GLP-1 analogue comprise nucleotide sequence as set forth in sequence ID nos 6 encoding the GLP-1 agonist peptide which is Liraglutide having amino acid sequence of sequence ID nos. 7.

In certain embodiemt the peptide may be optionally expressed with signal peptide or regulator/enhancer known to the skilled person.

In certain embodiemt the peptide may be optionally expressed with fusion partner or fusion tag in order to prevent the degradation of peptide. The fusion partner comprises amino acid sequence from 30 amino acid to 300 amino acid.The fusion partner comprises amino acid sequence selected from about 50 amino acid, 100 amino acid, about 136 amino acid, about 175 amino acid, about 250 amino acid, 300 amino acid, preferably about 136 amino acid.Fusion tag may be selected from but not limiting to Histidine-tag, glutathione-s-transferase (GST), Maltose binding protein, NusA, thioredoxin (TRX), polyhistidine (HIS), small ubiquitin-like modifier (SUMO) and ubiquitin (Ub) and staphylokinase (SAK) gene.In preferred embodiment the fusion tag is SAK gene. The detail use of SAK gene as fusion tag with protein of interest is disclosed in US8853380 which is incorporated herein as reference.

In some embodiment the bicistronic expression system further comprises selection marker which is selected from ampicillin, kanamycine, preferably ampicillin.

In another embodiment the present invention provides a process for producing a protein of interest comprising the steps of:

(i) transforming the host cell with a single vector essentially consisting of dual cistronic expression system;

(ii) culturing the transformed cell in suitable medium to express protein of interest, wherein the first and second cistron expresses the protein of interest in inclusion bodies;

(iii) performingsolubilizationof the inclusion bodies;

(iv) performing refolding of the protein of interest.

In embodiment the bicistronic expression system is transfected in to the suitable bacterial host cell in order to express the protein of interest. The suitable bacterial host cell is *E.coli*in which the protein of interest is expressed in the form of inclusion bodies. Inclusion bodies are the insoluble substance formed in the periplasm or cytoplasm of E.coli.Inclusion bodiesmay be isolated, solubilized and protein of interest may be recovered in active form by the techniques well known in the art.

In an embodiment, Fab heavy and light chainsof antibodies or other antibody fragments such as scFv, Diabodies, Triabodies, Tetrabodies, Bis-scFv, Minibodies Fab_2 (bispecific), Fab3 (trispecific)wereexpressed as insoluble inclusion bodies in the periplasmic space of *E. coli* by constructing two independent cistrons in a single vector having two different promoters, arabinose and T7 promoters. The two different promoters, i.e., T7 promoter and arabinose promoter helped in the expression of heavy and light chains of Fab molecule, respectively. The antibody heavy and light chains were produced as non-functional inclusion bodies in the bacterial cell, i.e, *E. coli* which are subsequently extracted, refolded and purified.

In an embodiment of the invention, the cistron comprises as such that each gene (heavy and light chain) would have its own promoter and terminatorin a single vector. The heavy chain was cloned under the control of T7 promoter while light chain was cloned under the control

of arabinose promoter. Both the chains were preceded by signal sequence pelB tag for obtaining the product in the periplasmic space of the bacterial membrane.

The advantage of the bicistronic expression system is that both arabinose and T7 being strong promoters, high expression of both light and heavy chains are obtained from a single fermentation run instead of separate fermentations with light and heavy chain clones.The dual-cistronexpression system makes it simpler to characterize and maintain a single cell bank instead of separate cell banks for light and heavy chain clones. Moreover, the inclusion bodies thus obtained are relatively pure when extracted from the periplasmic space of the bacterial cells. The high level of expression and much purer forms of the light and heavy chains obtained as inclusion bodies are relatively easier to fold into functional Fab *ex vivo*, thereby significantly incresing the yield of the product.

Another advantage of the system is that a protein of interest may be cloned and expressed under arabinose and T7 promoters and expression level of the protein may be increased significantly.

The examples disclosed below are only for illustrative purpose of the invention and are not intended to be limiting.

Example 1: Cloning of heavy chain in pET21a vector

The DNA sequence used for cloning of heavy and light chain of Fab fragmentsis given in sequence IDnos 1 and 2, respectively.The heavy chain insert was amplified from synthetic DNA using gene specific primers.Primersare designed according to methods well known in the art. The heavy chain PCR product was then digested with *NdeI-HindIII* enzymes and ligated to pET21a vector digested with the same enzymes.. The clones were screened by colony PCR and confirmed by restriction analysis. The resultant clone was designated as

pET21a-HC. The recombinant vector was introduced into BL21A1 cell line and checked for expression of heavy chain.

Example 2: Cloning of light chain in pBAD24M vector

The light chain insert was amplified from synthetic DNA using gene specific primers. Primers are designed according to methods well known in the art .The amplified light chain was digested with *NdeI-HindIII* enzymes and ligated to digested pBAD24M vector (available inthe laboratory) at same sites. The clones were screened by colony PCR and confirmed by restriction analysis. The resultant clone was designated as pBAD24M-LC. The recombinant vector was introduced into BL21A1 cell line and checked for expression of light chain.

Example 3: Construction of two independent cistrons in same vector

Primers were designed to amplify light chain along with arabinose promoter, terminator and araCgene. Primers are designed according to methods well known in the art.The primers added *BglII* linker to the amplified product. The pET21a vector had single *BglII* site upstream of the T7 promoter. The light chain expression cassette was amplified from the template pBAD24M with the vector specific primers and cloned into pET21a-HC clone at *BglII* site. The clone was confirmed by restriction digestion and sequencing. The final clone was designated as pET21a-HC-LC and suitable clone short listed based on expression. The clone map of the pET21a-HC-LC is presented in Figure 2.

The clone thus generated contains all the segments required for independent regulation and expression of both heavy and light chains.

Example 4: Expression analysis

*E. coli*BL21 A1 cell line was used as expression host. Apart from BL21 A1, BL21 DE3 or any other cell line containing T7 promoter in the genome is used. BL21 A1 cells were transformed using the above selected clone along with pET21a-HC and pBAD24M-LC as

controls. The Heavy chain was induced by IPTG while the light chain was induced by arabinose. The inducer concentration was 13 mM arabinose and 1 mM IPTG and the induction was done when the culture OD_{600} was ~1. The cells were harvested 4 hr post induction. The study was done in shake flasks. The harvest obtained was bead lysed and centrifuged to separate soluble and insoluble fractions. The samples were loaded on 12% SDS PAGE gels to check the expression. The SDS PAGE gel analysis is shown in Figure 3. Reduced ranibizumab was loaded in Lane 5 offFigure 3 to confirm expression of reduced light and heavy chains.

The SDS PAGE analysis showed expression of both chains in the insoluble pellet fraction and the same was confirmed by RP-HPLC analysis wherein the retention times of light and heavy chains of the reference product corresponded to the retention times of the in-house product. The controls used were reduced Fab molecule (reference product), and products of pET21a-HC clone and pBAD24M clone. Thus, the expression of both the heavy and the light chain from a single clone was confirmed. The RP-HPLC analysis is shown in Figures 4and 5. In RP-HPLC, solubilized and reduced IBs of the dual cistron clone were compared with reduced ranibizumab (RMP) and clones separately expressing heavy and light chainsi. e. pET21-HC and pBAD24MLC.

The retention time (RT) of principal peak of solubilized IB of pBAD24MLC expressing only light chain matches with the RT of light chain of reduced RMP. An impurity peak at RT 13 mins matches the retention time of heavy chain, which was indicative of similar hydrophobicity.The impurities therefore were characterized by LC-MS/MS and were finally annotated as host cell protein OMP C and light chain with uncleaved leader sequence at RT 17 mins. The heavy chain expressed by pET21a-HC matches with the reference standard heavy chain. The profile also indicated a post peak at RT 19 mins which was characterized as heavy chain with uncleaved leader sequence. The dual cistronic clone pET21a_HC_LC that expresses both LC and HC have 2 main peaks that have equivalent retention times as that of

the LC and HC of reference standard. But, since the OMP C co-eluted with heavy chain, the reversed phase method of testing had to be resolved better and this is presented in the Fig. 4.

The existing method on Zorbax C8 RP column was modified to Aeriswidepore C8 and the co-eluting species were resolved. The solubilized IB of pET21a_HC_LC on Aeriswidepore C8 exhibited a distinct LC, HC and OMP C peaks enabling the identity and precise quantification of individual subunits in IB as evident in Figure 5.

Although certain embodiments and examples have been described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments and examples without departing from the teachings thereof.

Example no 5: Cloning of small peptide (Liraglutide) with staphylokinase (SAK) fusion tag in pET24a vector

The SAK and Liraglutide genes were amplified from synthetic DNA using gene specific primers. Primers are designed according to methods well known in the art and PCR products were digested with*NdeI-BamHI and BamHI-HindIII* enzymes and ligated to digested pET24a vector at *NdeI-HindIII* sites. The clones were screened by colony PCR and confirmed by restriction analysis. The resultant clone was designated as pET24a-SAK-Lira.

Example 6: Cloning of small peptide (Liraglutide) with staphylokinase fusion tag in pBAD24M vector

As given in example no 6, Liraglutide with SAK tag was cloned into pBAD24M vector. The clone was designated as pBAD24M-SAK-Lira.

Example no 7:Construction of two independent cistrons in same vector with both cistrons expressing SAK-Lira fusion peptide

The clone design strategy used in example no. 3 was used to construct dual cistron clone of Liraglutide, wherein SAK-Lira fusion gene alongwith arabinose expression cassette was

amplifed from pBAD24M-SAK-Lira clone and cloned into pET24a-SAK-Lira clone to construct dual cistron construct. The clone was labelled as pET-ara-SAK-Lira

Example no 8: Expression analysis of dual cistron clone with SAK-Lira fusion protein.

*E. coli*BL21 A1 cell line was used as expression host. Apart from BL21 A1, BL21 DE3 or any other cell line containing T7 promoter in genome is used. BL21 A1 cells were transformed using the above single and dual cistron constructs. The clones were induced by IPTG and arabinose. The inducer concentration was 13 mM arabinose and 1 mM IPTG and the induction was done when the culture OD_{600} was ~1.The cells were harvested 4 hr post induction. The study was done in shake flasks. The harvest obtained was bead lysed and centrifuged to separate soluble and insoluble fractions. The samples were loaded on 12% SDS PAGE gels to check the expression.

The SDS PAGE gel analysis clearly shows increased expression of SAK-Lira fusion protein in dual cistron clone (Figure 6 lane 2) as compared with single cistron pET24a-SAK-Lira clone (Figure 6 Lane 3).

CLAIMS

1. A dual cistronic expression system comprising:
   a) first cistron comprising a promoter operably linked with polynucleotide sequence encoding protein of interest;
   b) second cistron comprising a promoter operably linked with polynucleotide sequence encoding protein of interest;
   wherein the first and second cistrons are positioned in single vector and express a polynucleotide sequence encoding the protein of interest as inclusion bodies formed in host cell.
2. The dual cistronic expression system as claimed in claim 1 wherein the first cistron and second cistron comprise a promoter selected from T7 promoter, arabinose promoter phoA, tac, lpp, lac-lpp, lac, trp and trc.
3. The dual cistronic expression system as claimed in claim 1 or claim 2 wherein the first and second cistron comprises a different promoter.
4. The dual cistronic expression system as claimed in claim 2 wherein the first cistron comprises a T7 promoter or arabinose promoter.
5. The dual cistronic expression system as claimed in claim 2 wherein the second cistron comprises a T7 promoter or arabinose promoter.
6. The dual cistronic expression system as claimed in claim 1 wherein the protein of interest is selected from proteins and peptides.
7. The dual cistronic expression system as claimed in claim 6 wherein the proteins are selected from GCSF, EPO, FSH, IFN, PTH (1-84 aa), Insulin, LH, RH, antibodies or fragments thereof.
8. The dual cistronic expression system as claimed in claim 7 wherein the antibody fragments are selected from light chain of antibody, heavy chain of antibody, scFv, FAB, Diabodies, Triabodies, Tetrabodies, Bis-scFv, Minibodies, Fab2 (bispecific) antibodies and Fab3 (trispecific) antibodies.

9. The dual cistronic expression system as claimed in claim 8 wherein the antibody fragment is Fab.
10. The dual cistronic expression system as claimed in claim 9 wherein the Fab fragment of antibody is Ranibizumab.
11. The dual cistronic expression system as claimed in claim 6 wherein the peptides are selected from Liraglutide, exanetide, PTH (1-34aa) and teduglutide.
12. The dual cistronic expression system as claimed in any one of the preceding claim comprising:

a) firstcistron comprising T7 promoter operably linked with polynucleotide sequence encoding heavy chain of antibody;

b) secondcistron comprising arabinose promoter operably linked with polynucleotide sequence encoding light chain of antibody;

wherein the first and second cistrons are positioned in single vector and express the heavy chain and light chain of the antibody as inclusion bodies formed in host cell.

13. The dual cistronic expression system as claimed in claim 12 wherein the heavy chain of antibody comprises the nucleotide sequence as set forth in sequence ID no 1 or the amino acid sequence as set forth in sequence ID no 3.
14. The dual cistronic expression system as claimed in claim 12 wherein the light chain of antibody comprises the nucleotide sequence as set forth in sequence ID no 2 or the amino acid sequence as set forth in sequence ID no 4.
15. The dual cistronic expression system as claimed in claim 12 wherein the heavy chain and light chain are cloned in suitable ratio having the heavy chain is equal or higher than light chain to obtain equimolar expression of heavy and light chain.
16. The dual cistronic expression system as claimed in any one of the preceding claim wherein the heavy and light chain of antibody have affinity to VEGF.
17. The dual cistronic expression system as claimed in any one of the preceding claim comprising:

a) firstcistron comprising T7 promoter operably linked with polynucleotide sequence encoding peptide

b) secondcistron comprising arabinose promoter operably linked with polynucleotide sequence encoding peptide;

wherein the first and second cistrons are positioned in single vector and express the peptide as inclusion bodies formed in host cell.

18. The dual cistronic expression system as claimed in claim 17 wherein the peptide is selected from GLP-1 analogue, GLP-2 analogue.

19. The dual cistronic expression system as claimed in claim 17 wherein the peptide comprise nucleotide sequence as set forth in sequence ID no 6 or the amino acid sequence as set forth in sequence ID no 7.

20. The dual cistronic expression system as claimed in claim 18 wherein the GLP-1 agonist peptide is Liraglutide.

21. The dual cistronic expression system as claimed in any of preceding claim wherein peptide or protein is expressed in combination with fusion tag.

22. The dual cistronic expression system as claimed in claim 21 wherein the fusion tag is staphylokinase (SAK) gene comprises nucleotide sequence as set forth in sequence ID 8.

23. The dual cistronic expression system as claimed in any one of the preceding claim further comprises an optional one or more elements selected from the group consisting of polynucleotide sequence encoding regulator to enhance the expression of protein of interest, polynucleotide sequence encoding signal peptide to regulate the expression of protein of interest in periplasmic space of host cell and selection marker.

24. The dual cistronic expression system as claimed in claim 23 wherein the regulator enhance the expression of light chain of antibody.

25. The dual cistronic expression system as claimed in claim 23 wherein the regulator is AraC gene.

26. The dual cistronic expression system as claimed in claim 23 wherein the signal peptide is pelB.

27. The dual cistronic expression system as claimed in claim 23 wherein the selection marker is ampicillin.

28. The dual cistronic expression system as claimed in any one of the preceding claim wherein the host cell is *E.coli*.

29. A process for producing a protein of interest comprising the steps of:

(i) transforming the host cell with a single vector essentially consisting of dual cistroicn expression system as claimed in any of the preceding claims;

(ii) culturing the transformed cell in suitable medium to express protein of interest, wherein the first and second cistron expresses the protein of interest in inclusion bodies;

(iii) performingsolubilization of the inclusion bodies;

(iv) performing refolding of the protein of interest.

30. The dual cistronic expression system as claimed in any one of the preceding claim comprises nucleotide sequence as set forth in sequence id no 19.

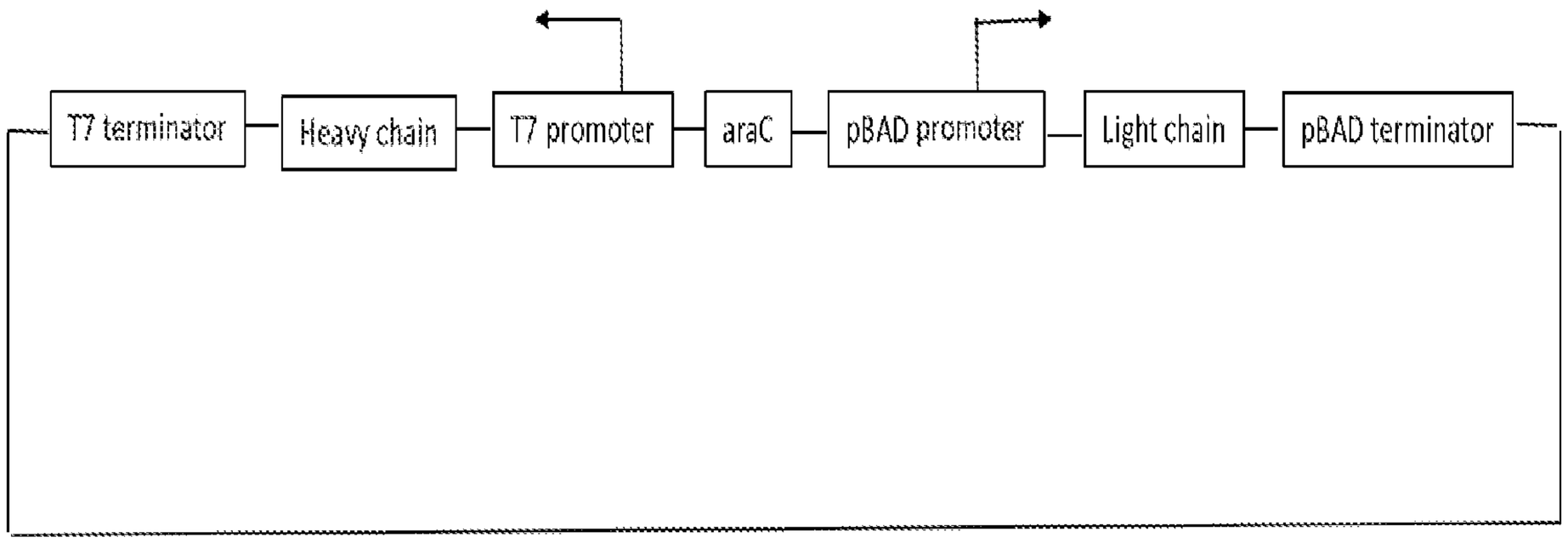

T7 terminator
Heavy chain
T7 promoter
araC
pBAD promoter
Light chain
pBAD terminator

Figure 1

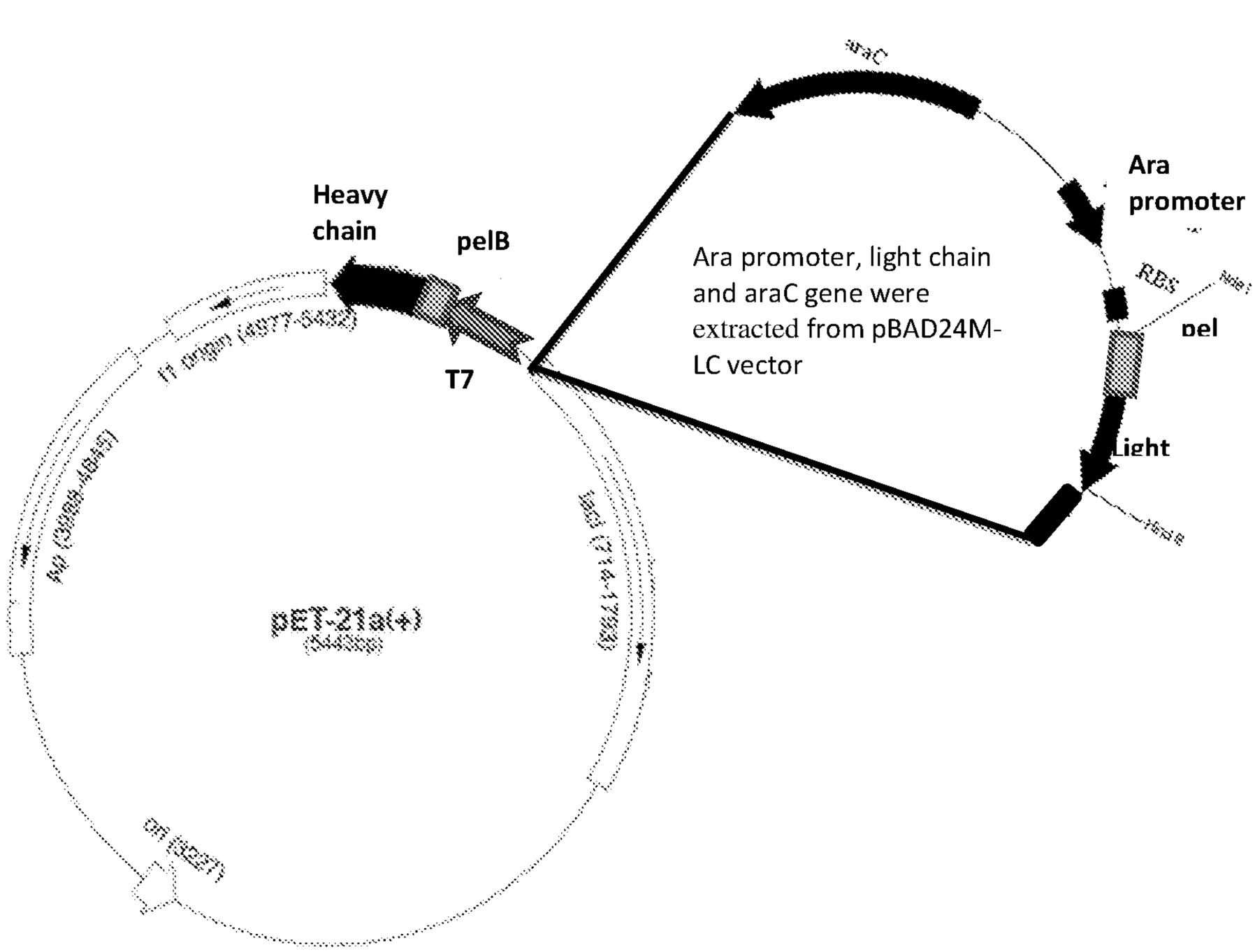
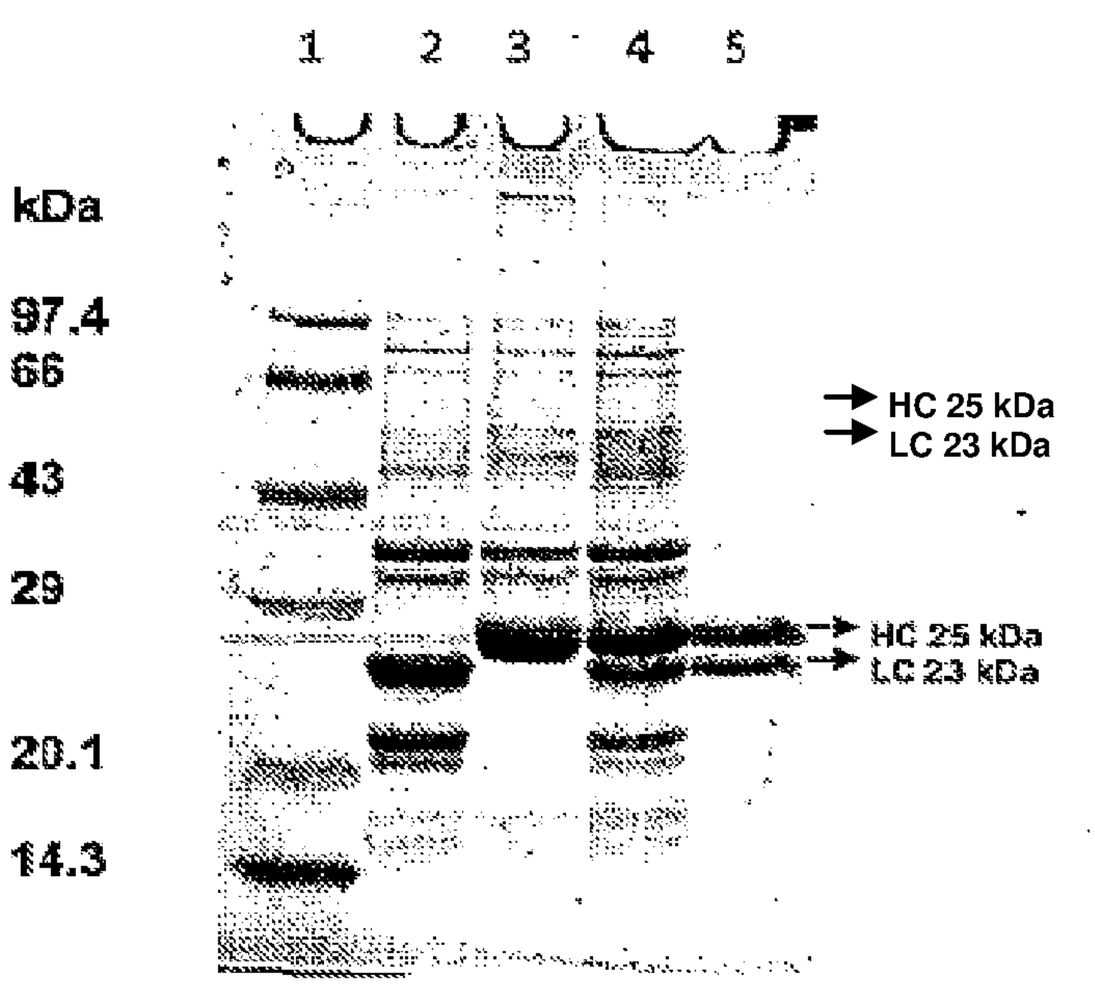

araC
Ara promoter
Heavy chain
pelB
Ara promoter, light chain and araC gene were extracted from pBAD24M-LC vector
RBS
pel
T7
Light
pET-21a(+)

Figure 2



Lane. No	Lane description
1	Protein molecular weight marker
2	Light chain pBAD/BL21 A1
3	Heavy chain pET21a/BL21A1
4	Dual cistron clone pET21a-HC-LC /BL21 A1
5	Ranibizumab standard

Figure 3

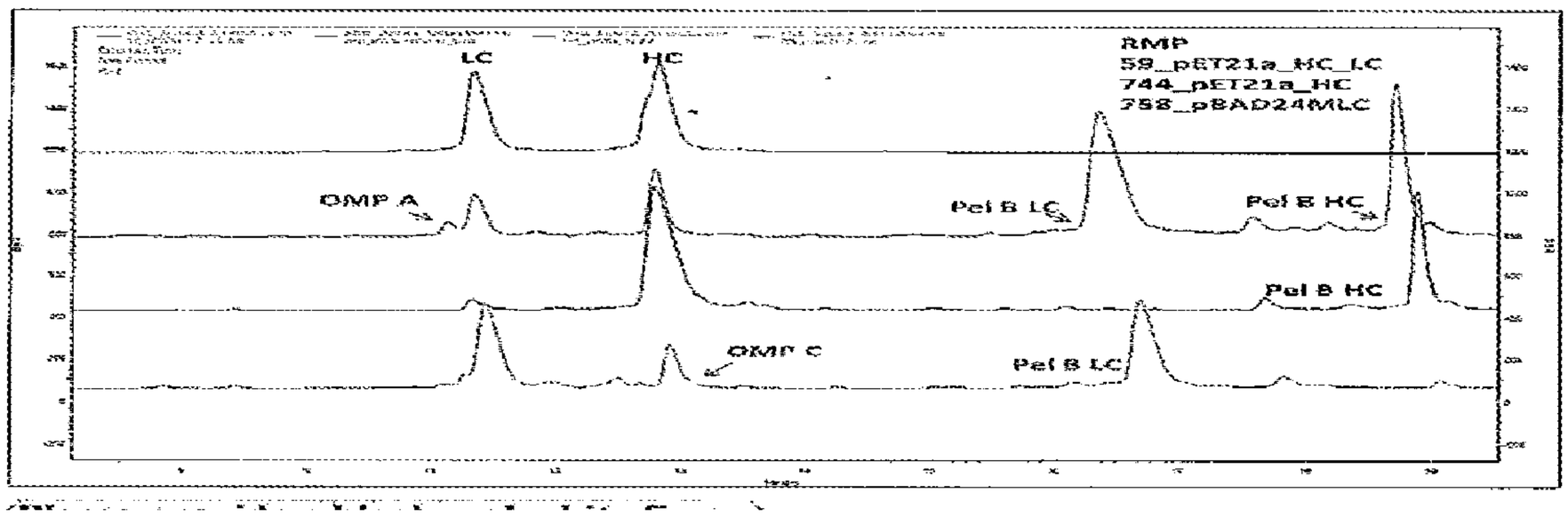

LC
HC
RMP
59_pET21a_HC_LC
744_pET21a_HC
758_pBAD24MLC
OMP A
Pel B LC
Pel B HC
Pel B HC
OMP C
Pel B LC

Figure 4

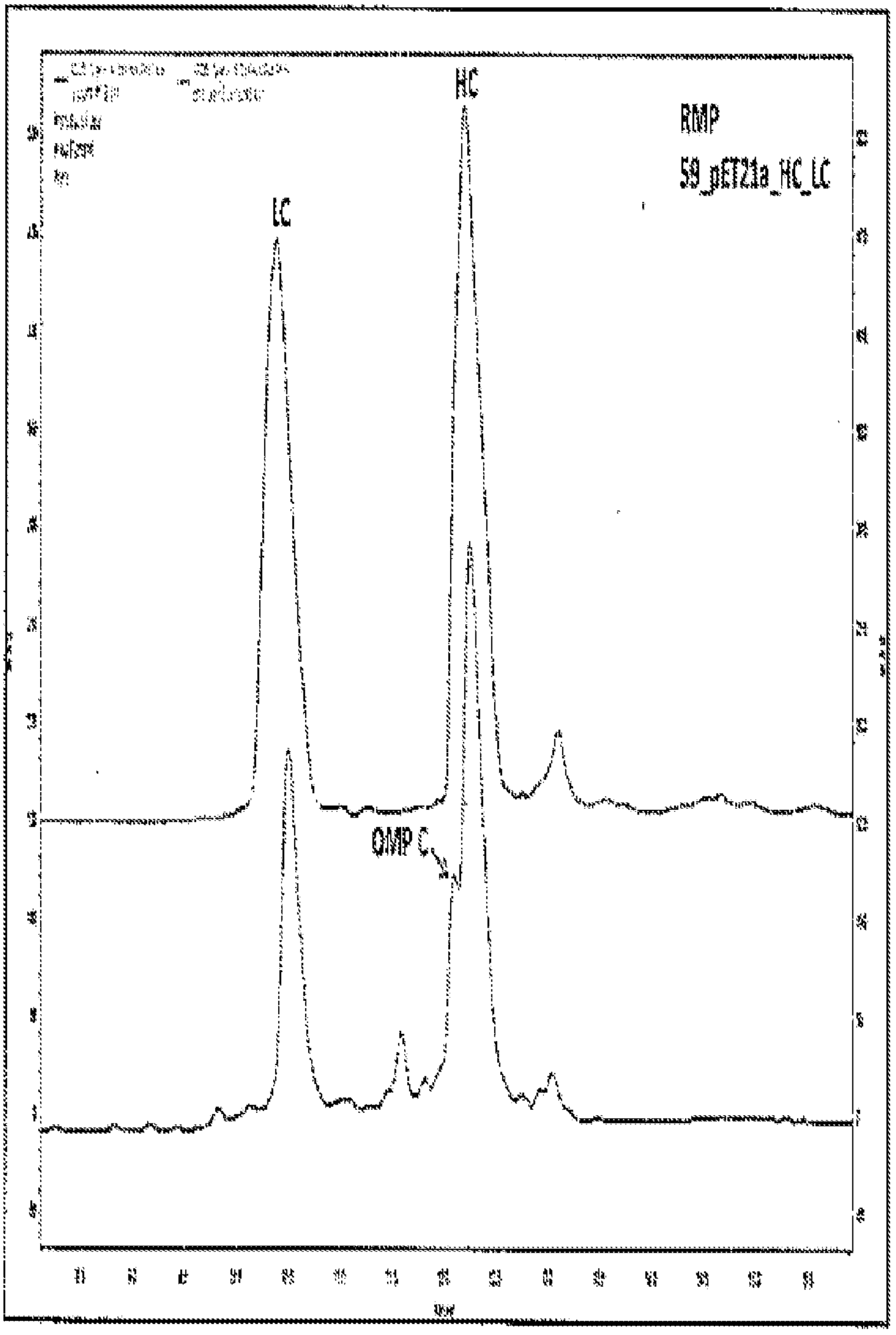
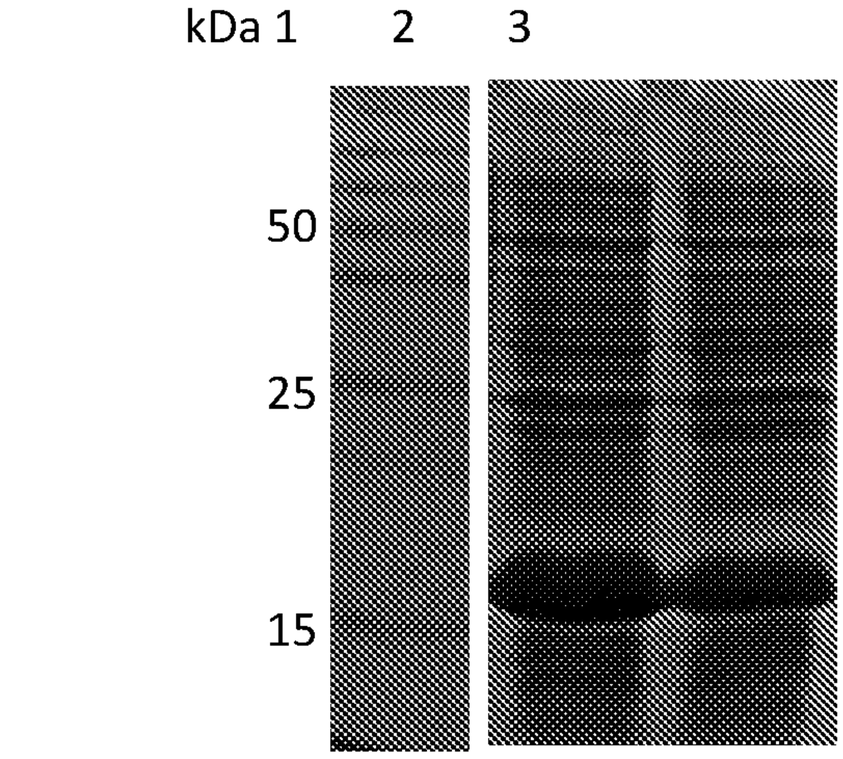


Figure 5



Lane. No	Lane description
1	Protein molecular weight marker
2	Dual cistron clone pET-ara-SAK-Lira /BL21 A1
3	Single cistron clone pET-SAK-Lira

Figure 6

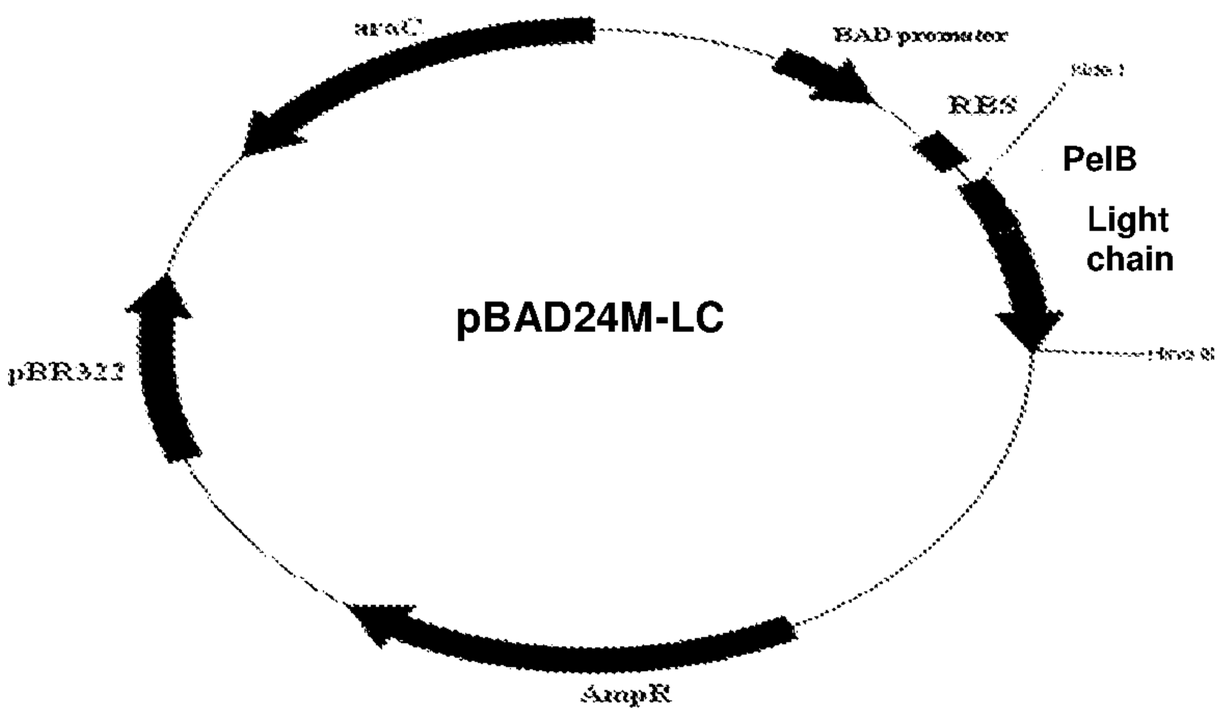
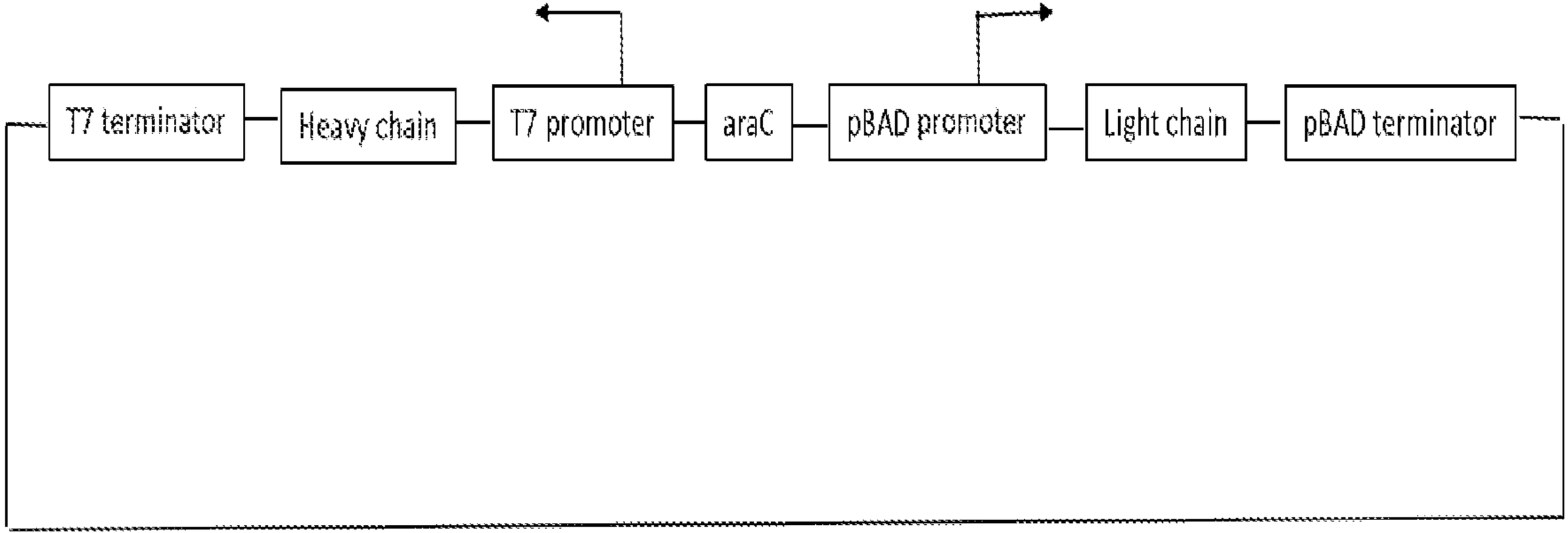


Figure 7


T7 terminator
Heavy chain
T7 promoter
araC
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Light chain
pBAD terminator

Figure 1