DIAGNOSTIC TEST AND TREATMENT/PREVENTION OF ALZHEIMER'S DISEASE

TECHNICAL FIELD OF THE INVENTION

The present invention relates in general to the field of Alzheimer's Disease, and more particularly, to a diagnostic method and compositions and methods for treatment and prevention of Alzheimer's Disease.

BACKGROUND OF THE INVENTION

Without limiting the scope of the invention, its background is described in connection with Alzheimer's Disease.

United States Patent No. 7,479,550, issued to United States Patent No. 7,479,550, issued to Rosenberg, et al., is entitled "Amyloid β gene vaccines." This invention includes compositions and methods for genetic vaccination with amyloid beta (Aβ) protein. The vaccine is said to provide effective treatment for neurodegenerative disease such as Alzheimer's disease. Vaccination methods are can be used to induce a Th2 type immune response directed to Aβ. This immune response is said to substantially reduce Aβ concentration and Aβ plaque size in an Alzheimer's model system. One challenge with the use of this system is the need to use two separate vectors known as the Gal4/UAS system. The Gal4/UAS system was effective in inducing an immune response against the Amyloid ABeta-42 peptide in a transgenic mouse model, resulting in inhibition of Amyloid ABeta-42 accumulation. However, the two-vector system, also called a binary vector system, uses two plasmid vectors, that impose a greater production burden, sterility issues and suboptimal use with patients. A single plasmid vaccine would be ideal for both production and application in the clinic.

United States Patent No. 4,816,388, issued to Sipe, et al., is entitled "Human prealbumin and related methods and products". Briefly, this patent is said to teach, in addition to recombinant human prealbumin, the use of human prealbumin cDNA in the diagnosis by hybridization methodologies of medical conditions with which variant forms of prealbumin are associated, namely, diagnosing Type I familial amyloid polyneuropathies by a restriction endonuclease assay with an enzyme which recognizes the nucleotide base sequence 5'-ATGCAT-3'.

United States Patent Application Publication No. 2014/0031245, filed by Khan, et al., is entitled Alzheimer's Disease-Specific Alterations of the ERK1/ERK2 Phosphorylation Ratio-Alzheimer's Disease-Specific Molecular Biomarkers (ADSMB). Briefly, this application is said to teach methods of diagnosing Alzheimer's Disease as well as to methods of confirming the presence or absence of Alzheimer's Disease in a subject. The present invention identifies a lead compound useful for the treatment of Alzheimer's Disease by contacting non-Alzheimer's cells with an amyloid beta peptide, stimulating the cells with a protein kinase C activator, contacting the cells with a test compound, and determining the value of an Alzheimer's Disease-

specific molecular biomarker. The application is also said to teach methods of diagnosing Alzheimer's Disease in a subject by detecting alterations in the ratio of specific phosphorylated MAP kinase proteins in cells after stimulation with a protein kinase C activator.

United States Patent Application Publication No. 2012/0192294, filed by Heneka, et al., is entitled "Inhibitors of the Nitration of Amyloid Beta Peptides and Their Uses in the Diagnosis and Treatment of Alzheimer's Disease." Briefly, this application is said to teach a method for identifying an inhibitor of the aggregation of amyloid-β peptide (Aβ), comprising the steps of a) contacting at least one Aβ-peptide and/or the nitrated forms thereof with at least one candidate inhibitor that potentially specifically binds to a region in said Aβ-peptide capable of being nitrated, and b) detecting said inhibitor specifically binding to said region in said Aβ-peptide through detecting a lack of or a reduced aggregation of said at least one Aβ-peptide. The present invention is further directed at improved methods for treating neuronal degradation and particularly Alzheimer's disease, based on said inhibitor. The present invention is further directed at methods for diagnosing the aggregation of Aβ-peptide in the context of neuronal degradation and particularly Alzheimer's disease.

SUMMARY OF THE INVENTION

The present invention includes a diagnostic test for Alzheimer's Disease based on the protein levels and/or RNA expression levels of the protein Heat Shock Protein 27 (HSP27) in patient samples such as tissue, fluids such as blood or other bodily elements from patients who had or were predisposed to disease such as Alzheimer Disease. The levels of HSP27 would be determined in the patient samples using ELISA, nucleic acid hybridization, Nano-BioSensor technology or other detection systems. The diagnostic test was then used to direct treatment or prevention of Alzheimer's Disease in potential patients and patients with a novel expression vector.

In one embodiment, the present invention includes a method for diagnosis and treatment and prevention of Alzheimer's Disease comprising: obtaining a biological sample from a subject suspected of having Alzheimer's Disease; determining the level of expression of HSP 27, wherein a statistically significant increase in HSP27 protein expression in the sample as compared to a sample from a non-Alzheimer's patient is indicative that the subject has Alzheimer's Disease; and modifying the treatment of the subject as a result of the detection of Alzheimer's Disease by providing the subject with standard therapy or a composition comprising a single DNA vector encoding the Aβ42 trimer peptide, wherein the expressed Aβ42 trimer peptide triggers an immune response to the Aβ42 peptide. In one aspect, the subject is a human. In another aspect, the HSP27 is human HSP27. In another aspect, the composition further comprises an Aβ42 peptide and the composition comprising the DNA vector and the Aβ42 peptide is injected intramuscularly without the need for a gene gun or gold particles. In another aspect, the level of HSP 27 is determined by measuring protein expression, and the method is selected from fluorescence detection,

chemiluminescence detection, electrochemiluminescence detection and patterned arrays, antibody binding, fluorescence activated sorting, detectable bead sorting, antibody arrays, microarrays, enzymatic arrays, receptor binding arrays, solid-phase binding arrays, liquid phase binding arrays, fluorescent resonance transfer, or radioactive labeling. In another aspect, the level of expression of HSP27 is determined at the nucleic acid level, and the method is selected from fluorescence detection, chemiluminescence detection, electrochemiluminescence detection and patterned arrays, reverse transcriptase-polymerase chain reaction, detectable bead sorting, microarrays, enzymatic arrays, allele specific primer extension, target specific primer extension, solid-phase binding arrays, liquid phase binding arrays, fluorescent resonance transfer, or radioactive labeling. In another aspect, the level of expression of HSP27 is higher than 85, 90, 95, 100, 110, 115, 120, 125, 130, 145, 150, 275, 300, or 315 ng/ml HSP27 in a blood sample. In another aspect, the level of expression of HSP27 is higher than 105, 130, 145, 150, 275, 300, or 315 ng/ml HSP27 in a blood sample. In another aspect, the expressed Aβ42 trimer peptide triggers a non-inflammatory IgG1 response and not an IgG2a or IgG2b response. In another aspect, the Aβ42 peptide and the DNA vector expressing the Aβ42 trimer peptide are effective to trigger an immune response to the Aβ42 peptide without an adjuvant.

Another embodiment of the present invention include a method to evaluate a candidate drug believed to be useful in treating Alzheimer's Disease, the method comprising: (a) measuring the level of expression of HSP27 from a sample obtained from an Alzheimer's Disease patient; (b) administering a candidate drug to a first subset of the patients, and a placebo to a second subset of the patients, wherein the candidate drug comprises a single DNA vector encoding an Aβ42 trimer peptide; (c) determining if the level of expression of HSP27 or the symptoms of Alzheimer's Disease decreased in the first set of patient as compared to the second subset of patients, wherein a statistically significant decrease is indicative that the candidate drug is useful for treating Alzheimer's Disease. In one aspect, the subject is a human. In another aspect, the drug candidate further comprises an Aβ42 trimer peptide and the DNA vector and the Aβ42 trimer peptide are injected intramuscularly without the need for a gene gun or gold particles. In another aspect, the HSP27 is human HSP27. In another aspect, the level of HSP 27 is determined by measuring protein expression, and the method is selected from fluorescence detection, chemiluminescence detection, electrochemiluminescence detection and patterned arrays, antibody binding, fluorescence activated sorting, detectable bead sorting, antibody arrays, microarrays, enzymatic arrays, receptor binding arrays, solid-phase binding arrays, liquid phase binding arrays, fluorescent resonance transfer, or radioactive labeling. In another aspect, the level of expression of HSP27 is determined at the nucleic acid level, and the method is selected from fluorescence detection, chemiluminescence detection, electrochemiluminescence detection and patterned arrays, reverse transcriptase-polymerase chain reaction, detectable bead sorting, microarrays, enzymatic arrays, allele specific primer extension, target specific primer extension, solid-phase binding arrays, liquid phase binding arrays, fluorescent resonance transfer, or radioactive labeling. In another aspect, the level of expression of HSP27 is higher than 85, 90, 95, 100, 110, 115, 120, 125, 130, 145, 150, 275, 300, or 315 ng/ml HSP27 in a

blood sample. In another aspect, the level of expression of HSP27 is higher than 105, 130, 145, 150, 275, 300, or 315 ng/ml HSP27 in a blood sample. In another aspect, the DNA vector is a single DNA vector.

Yet another embodiment of the present invention includes compositions, methods, pharmaceuticals, methods of making, using and compositions manufactured to treat or prevent Alzheimer's Disease that include a single vector comprising: a single nucleic acid that comprises in the following order a viral gene leader sequence, a Aβ42 trimer sequence, and an endosomal targeting sequence. In one aspect, the viral gene leader sequence is an adenovirus E3 gene leader sequence. In another aspect, the vector further comprises a CMV promoter upstream from the nucleic acid. In another aspect, the vector comprises SEQ ID NO: 1. In another aspect, the wherein the endosomal targeting sequence is, e.g., DXXLL (SEQ ID NO: 2), or can be obtained from the human invariant (Il) chain. In another aspect, the vector is PV1-H3. In another aspect, the vector is PV1-H3 is used to treat or prevent Alzheimer's Disease.

Yet another embodiment of the present invention includes a composition for ameliorating the symptoms of Alzheimer's Disease comprising an Aβ42 trimer peptide and a DNA vector encoding the Aβ42 trimer peptide in an amount sufficient to ameliorate the symptoms of Alzheimer's Disease. In one aspect, the composition is provided without an adjuvant. In another aspect, the composition triggers a predominantly Th2 response. In another aspect, the Aβ42 trimer peptide and the DNA vector are injected intramuscularly without the need for a gene gun or gold particles. In one aspect, the DNA vector is a single DNA vector. In another aspect, the peptide comprises SEQ ID NO: 3. In another aspect, the vector comprises SEQ ID NO: 1. In another aspect, the composition consists essentially of the vector of SEQ ID NO: 1 and the peptide of SEQ ID NO: 3.

In yet another embodiment, the present invention includes a composition for ameliorating the symptoms of Alzheimer's Disease comprising both an Aβ42 peptide and a DNA vector that expresses an Aβ42 trimer peptide in an amount sufficient to ameliorate the symptoms of Alzheimer's Disease, wherein the Aβ42 peptide and the DNA vector that expresses the Aβ42 trimer peptide are both injected intramuscularly without the need for a gene gun or gold particles (and a use of the same), wherein the composition triggers an immune response to the Aβ42 peptide. In one aspect, the expressed Aβ42 trimer peptide and the DNA vector are provided without an adjuvant. In another aspect, the DNA vector is a single DNA vector. In another aspect, the composition leads to a predominantly Th2 response. In another aspect, the peptide comprises SEQ ID NO: 3. In another aspect, the vector comprises SEQ ID NO: 1. In another aspect, the composition consists essentially of the vector of SEQ ID NO: 1 and the peptide of SEQ ID NO: 3.

In another embodiment, the present invention includes a method for the treatment or prevention of Alzheimer's Disease comprising injecting a composition that includes both an Aβ42 peptide and a DNA vector that expresses an Aβ42 trimer peptide, wherein the Aβ42 peptide and the DNA vector are adapted for injection intramuscularly without the need for a gene gun or gold particles (and a use of the same), wherein

the composition triggers an immune response to the Aβ42 peptide. In another aspect, the injection triggers a non-inflammatory IgG1 response. In another aspect, the Aβ42 peptide and the DNA vector are provided without an adjuvant. In another aspect, the DNA vector is a single DNA vector. In another aspect, the composition leads to a predominantly Th2 response. In another aspect, the Aβ42 peptide comprises SEQ ID NO: 3. In another aspect, the vector comprises SEQ ID NO: 1. In another aspect, the composition consists essentially of the vector of SEQ ID NO: 1 and the peptide of SEQ ID NO: 3.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures and in which:

Figure 1 is a graph that shows the DNA binding to gold particles. The optimal ratio of DNA to the gold is 4.5 ug DNA (p4u-Ab42 trimer) with 1 mg gold. In this ratio, about 3.8 ug Ab42 trimer DNA can be bind to 1.5 mg gold per cartridge (Bullet) plus 20% CMVi-Gal4 DNA as additional.

Figure 2 is a schematic presentation of single plasmid vector for DNA vaccine against Alzheimer's disease. The Amyloid ABeta-42 trimer gene was cloned between a CMV (pCMV) promoter upstream and SV40 PolyA downstream.

Figure 3 shows that the single plasmid of the present invention is 2X more active than a binary system.

Figure 4 shows an antibody isotyping of the antibody generated by the single plasmid PV1-H3 – which is a non-inflammatory profile.

Figure 5 is a graph that shows the results from 4 muscle injections (once a week (20 ug) with trimer DNA +10 ug Aβ peptide (or separate injection) and tested the antibodies at 6 weeks. It was found that DNA+Peptide without adjuvant elicit a better immune response.

Figure 6 is a graph that shows the results from 4 muscle injections 4 times (once a week) muscle injection (20 ug Trimer DNA +10 ug Aβ peptide), the serum was tested for Abeta isotype antibodies at 6 weeks with ELISA method. It was found that DNA+Peptide without adjuvant elicited a better immune response compare to peptide alone. Higher isotype antibodies level achieved in DNA+Peptide, but both group induced Th1 and Th2 reaction with predominantly Th2 (IgG1 and IgG2a) bias.

DETAILED DESCRIPTION OF THE INVENTION

While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are

merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as "a", "an" and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

The term "Amyloid ABeta-42" taught herein refers to the nucleotides encoding the Amyloid ABeta-42 peptide variant taught herein that is a portion of the entire vector set forth (SEQ ID NO: 1), and that has amino acid sequence SEQ ID NO: 2.

The terms "a sequence essentially as set forth in SEQ ID NO: (#)", "a sequence similar to", "nucleotide sequence" and similar terms, with respect to nucleotides, refers to sequences that substantially correspond to any portion of the sequence identified herein as SEQ ID NO: 1. These terms refer to synthetic as well as naturally derived molecules and includes sequences that possess biologically, immunologically, experimentally, or otherwise functionally equivalent activity, for instance with respect to hybridization by nucleic acid segments, or the ability to encode all or portions of Amyloid ABeta-42 or Amyloid ABeta-42 activities. Naturally, these terms are meant to include information in such a sequence as specified by its linear order.

The terms "a sequence essentially as set forth in SEQ ID NO: 2", "a sequence similar to", "amino acid sequence" and similar terms, with respect to amino acids, refers to peptides, polypeptides, proteins, fragments, fusions, derivatives and alterations thereof that substantially correspond to the sequences of SEQ ID NO: 2. These terms refer to synthetic as well as naturally derived molecules and includes sequences that possess biologically, immunologically, experimentally, or otherwise functionally equivalent activities, for instance, segments of amino acids which possess immunological activity as an antigenic determinant. Naturally, these terms are meant to include information in such a sequence as specified by its linear order.

The term "gene" is used to refer to a functional protein, polypeptide or peptide-encoding unit. As will be understood by those in the art, this functional term includes genomic sequences, cDNA sequences, or fragments or combinations thereof, as well as gene products, including those that may have been altered by the hand of man. Purified genes, nucleic acids, protein and the like are used to refer to these entities when identified and separated from at least one contaminating nucleic acid or protein with which it is ordinarily associated.

As used herein, the term "vector" is used in reference to nucleic acid molecules that transfer DNA segment(s) from one cell to another. The vector may be further defined as one designed to propagate the

sequences, or as an expression vector that includes a promoter operatively linked to the Amyloid ABeta-42 gene sequence taught herein, or one designed to cause such a promoter to be introduced. The vector may exist in a state independent of the host cell chromosome, or may be integrated into the host cell chromosome.

The term "host cell" refers to cells that have been engineered to contain nucleic acid segments for the Amyloid ABeta-42 gene taught herein, or altered segments, whether archeal, prokaryotic, or eukaryotic. Thus, engineered, or recombinant cells, are distinguishable from naturally occurring cells that do not contain recombinantly introduced genes through the hand of man.

As used herein, the term "endosomal targeting sequence" refers to an amino acid sequence that targets a polypeptide (or portion thereof) that when included in the polypeptide (e.g., fused or conjugated to the polypeptide), increases endosomal localization of the polypeptide. Endosomal targeting signals for directing molecules to endosomes are known in the art and the sequences can be incorporated in expression vectors such that fusion proteins will contain the endosomal targeting signal are produced, see e.g., Sanderson et al. (Proc. Nat'l. Acad. Sci. USA 92:7217-7221, 1995), Wu et al. (Proc. Nat'l. Acad. Sci. USA 92:11671-11675, 1995) and Thomson et al (J. Virol. 72:2246-2252, 1998), which describe endosomal targeting signals (including invariant chain Ii and lysosomal-associated membrane protein LAMP-1) and their use in directing antigens to endosomal and/or lysosomal cellular compartments. Thus, endosomal targeting sequences can include the entire sequence or only a small portion of a targeting sequence such as, e.g., human invariant chain, and can even be included in a pro-polypeptide that is removed one the polypeptide reaches the endosome. One of ordinary skill in the art can readily determine an endosomal targeting portion of a targeting molecule and use well-known molecular biology techniques to make a recombinant fusion protein that include the endosomal targeting sequence. Additional endosomal targeting sequences can be identified by one of ordinary skill in the art and tested for targeting to the HLA class II peptide presentation pathway using no more than routine experimentation.

HSP27. The twenty-seven kiloDalton heat shock protein (Hsp27) belongs to the small heat shock protein family, which are ATP-independent chaperones. The most important function of Hsp27 is based on its ability to bind non-native proteins and inhibit the aggregation of incorrectly folded proteins maintaining them in a refolding-competent state. Additionally, it has anti-apoptotic and antioxidant activities.

Alzheimer's disease (AD) is characterized by pathological lesions such as senile plaques (SP), cerebral amyloid angiopathy (CAA) and neurofibrillary tangles (NFT), predominantly consisting of the incorrectly folded proteins amyloid-β (Aβ) and tau respectively. The extracellular expression of Hsp27 has been observed in classic SP, and in astrocytes associated with both SP and CAA. Amyloid-β (Aβ) and tau proteins found within the pathological lesions induces neuronal loss and cognitive deficits and is believed to be a prominent cause of AD. Although a great amount of work has gone into studying Alzheimer's disease AD, there is currently no accurate or sensitive technique to determine the onset of AD.

The present inventors determined that early in the process of Alzheimer's disease (AD) the dead and dying cells within pathological lesions such as senile plaques (SP), cerebral amyloid angiopathy (CAA) and neurofibrillary tangles (NFT) release their cellular content which makes its way into the systemic circulation. Since Hsp27 makes up a high proportion of the pathological lesions, the inventors developed a simple, sensitive blood test for Hsp27 to determine the early onset of Alzheimer's disease (AD).

Using Heat Shock Protein 27 (HSP27) as a Biomarker.

The present invention includes a diagnostic test for Alzheimer Disease based on the protein levels and/or RNA expression levels of the protein Heat Shock Protein 27 (HSP27) in patient samples such as tissue, fluids such as blood or other bodily elements from patients who had or were predisposed to any disease including Cancer and Alzheimer Disease. The levels of HSP27 are determined in the patient samples using ELISA, nucleic acid hybridization, Nano-BioSensor technology or other detection systems.

HSP27 is a 27,000 dalton member of the Heat Shock Protein (HSP) family. The HSP proteins are ATP-independent chaperones, which work to maintain the integrity of protein structure such as folding of the protein. Perturbations to such structural protein integrity are associated with different disease states. One example is the incorrect folding of Aβ amyloid, which is associated with the early steps involved in Alzheimer's Disease.

A diagnostic test was developed to determine the levels of HSP27 proteins and/or expression levels of the HSP27 gene in blood, tissue or other body elements as an indication of the existence of or prediction of diseases such as Alzheimer's Disease, cancer and other diseases. An ELISA immunology test specific for HSP27 was employed to identify the HSP27 protein levels in patient samples and in samples from individuals which do not have the disease in question and the HSP27 protein levels are compared. If the samples from the patients with the disease show a statistically higher level of the HSP27 (or a lower level depending on the disease), then that could be the basis for a diagnostic test for that particular disease. In addition to comparing the levels of HSP27 proteins in disease (or pre-disease) samples and non-disease samples, the levels of the HSP27 mRNA in disease and non-disease samples can also be determined by hybridization using DNA or other nucleic acid probes. One example of such a methodology to improve diagnostic tests is Nano-BioSensor technology (one example is Guided-Mode Resonance Sensor Technology), which permits detection of the HSP27 protein or mRNA without the need for tags such as radio-isotopes or chemical tags such as Biotin and permit reading the results in real time.

Hsp27 in blood samples is indicative of the early onset of Alzheimer's disease (AD). To test this hypothesis we obtained blood samples from 8 patients recently determined to have early onset Alzheimer's disease (AD) as determined from clinical records and 5 normal age and sex matched subjects. Plasma proteins were recovered from the blood and tested for the concentration of phosphorylated Hsp27 (pHsp27) using the classical sandwich enzyme linked immunosorbant assay (ELISA). Briefly, blood was drawn from

patients and added to tubes containing EDTA, centrifuged and the plasma was recovered, aliquoted and stored at -80°C. The total protein content of each aliquot was determined by Bradford analysis using bovine serum albumin as a standard. The samples were then admixed with 1% Lubrol WX for 10 minutes at 4°C with gentle rocking and pHsp27 content measured by standard sandwich ELISA. Briefly, 96-well microtitre plates (Nunc Immunoplate Maxisorp; Life Technologies) were coated with murine monoclonal anti-human pHsp27 in carbonate buffer, pH 9.5 (2 μg/mL) overnight at 4°C. Plates were then washed with PBS containing 1% Tween-20 (PBS-T) and blocked by incubation with 1% bovine serum albumin in PBS-T. Supernatant was added and bound pHsp27 was detected by the addition of rabbit polyclonal anti-pHsp27 antibody. Bound polyclonal antibody was detected with alkaline phosphatase-conjugated murine monoclonal antibody to rabbit immunoglobulins (Sigma Chemical Co), followed by p-nitrophenyl phosphate substrate (Sigma Chemical Co). The resultant absorbance was measured at 405 nm with a BioRad Benmark Plus plate reader. Standard dose-response curves were generated in parallel with pHsp27 (0 to 20,000 ng/mL; StressGen), and the concentrations of pHsp27 were determined by reference to these standard curves with ASSAYZAP data analysis software (BIOSOFT). The inter-assay variability of the pHsp27 immunoassays was <10%. The results demonstrate that there was a significant increase in pHsp27 in the plasma of 8/8 patients with Alzheimer's disease (AD) as compared to the 5 normal subjects (Table 1).

Table 1. Measurement of Hsp27 levels in patients with early onset Alzheimer's disease (AD).

Patient number	Mean Hsp72 concentration (ng/ml ± SD)a
Control #12-581	60 ± 10
Control #12-254	85 ± 15
Control #12-542	65 ± 13
Control #12-154	45 ± 11
Control #12-488	82 ± 15
AD #12-658	125 ± 12*
AD #12-325	145 ± 13*
AD #12-782	225 ± 28*
AD #12-489	138 ± 15*
AD #12-770	149 ± 25 *
AD #12-333	308 ± 50*
AD #12-411	149 ± 15*
AD #12-807	108 ± 23*

Data are plasma pHsp27 concentrations from control (normal subjects) and patients with Alzheimer's disease (AD) measured using the classical pHsp27 ELISA as described in detail in the Materials and Methods section. Data is the mean concentration of pHsp27 (ng/ml ± SD) and is the

sum of three independent experiments performed in quadruplicates. *, $p<0.001$ vs control (normal subjects).

The results show a mean HSP27 concentration (ng/ml) of 60, 85, 65, 45, 82 in the five non-Alzheimer Disease blood samples and HPS27 concentration (ng/ml) of 125, 145, 225, 138, 149, 308, 149, 108 in the eight Alzheimer Disease blood samples. Hence, there is a statistically significant ($p<0.001$) increase in the level of HSP27 in the blood of Alzheimer Disease patients compared to non-Alzheimer Disease patient blood. Therefore this is a disclosure that a Diagnostic test based on the levels of HSP27 in blood samples from individuals could indicate the presence of Alzheimer Disease.

A comparable study with a larger number of samples from a larger number of Alzheimer's Disease and non-Alzheimer's Disease individuals. In addition, determination can be made of the HSP42 levels in family members who carry PS1 or PS2 gene defects but have no symptoms of Alzheimer Disease. Individuals who have PS1 or PS2 gene defects have greater than 90% chance of getting Alzheimer Disease starting at an age of approximately 45-50 as opposed to age 65-70 or older, which is common for other forms of the disease. High levels of HSP27 in PS1 or PS2 asymptomatic patients may proceed to develop Alzheimer's Disease, as such, the levels of HSP27 are predictive of Alzheimer's Disease before symptoms; hence a Diagnostic Test showing HSP27 levels can be predictive and could be used to screen the blood of the general population for Alzheimer's Disease.

Once diagnosis is made using the present method, or other methodologies, including standard Alzheimer's Disease clinical testing, the present invention also includes a novel nucleic acid vector with enhanced delivery to target cells, and enhanced expression of functional Aβ42 trimer peptide.

Construction of the plasmid.

The present inventors have constructed a Single Plasmid Vector that has the three copies of the Amyloid ABeta-42 gene, cloned between the CMV promoter upstream and SV40 polyA downstream (Figure 2). Surprisingly, the Single Plasmid Vector PV1-H3, is more active and induces two-fold more antibody against the Amyloid ABeta-42 compared to the Two Vector System. This was surprising because it was assumed that the activity would be about the same as the initial two-vector system. However, the present invention finds the advantage of being a single vector system making manufacturing, regulatory approval, and clinical utility, less complex in addition to the advantage of demonstrating more activity.

Figure 2 is a schematic presentation of single plasmid vector for DNA vaccine against Alzheimer's disease. The Amyloid ABeta-42 trimer gene was cloned between a CMV (pCMV) promoter upstream and SV40 PolyA downstream.

Figure 3 shows that the single plasmid of the present invention is 2X more active than a binary system. Briefly, the single plasmid PV1-H3 was found to induce about 2X more antibody against Amyloid

ABeta-42 when 8 applications are used (33 ug/ml of Anti-Abeta42 antibody by the Single Vector PV1-H3 compared to 16 ug/ml of the Anti-Abeta42 antibody by the Binary System P4u-H3).

Figure 4 shows an antibody isotyping of the antibody generated by the single plasmid PV1-H3 – which is a non-inflammatory profile.

It was also found, surprisingly, that the PV1-H3 Single Plasmid of the present invention generated predominately IgG1 Antibody and minor amounts of IgG2a and IgG2b. The IgG1 antibody is not involved in the inflammatory response. Previous studies using the Amyloid ABeta-42 Peptide itself as a Vaccine, not the gene, induced equal amounts of IgG1 and IgG2a which resulted in an inflammatory response. These results confirm that the PV1-H3 generates predominantly IgG1 which is not inflammatory.

A highly efficient Single Vector, PV1-H3, has been created by the present inventors, which induces two fold higher levels of Antibody against Amyloid ABeta-42 Peptide than the two vector system and the Antibody generated is 90% IGg1 which is characteristic of a non-inflammatory response.

The Aβ42 trimer genes were chemically synthesized and cloned into the immunization vector system.

1. A set of complementary oligonucleotides of the A.42 DNA sequence were designed using the DNA builder program and custom synthesized (Sigma, St. Louis, MO).
2. These oligonucleotides were designed after the respective Aβ42 amino acid sequence using multiple codons for a particular amino acid allowing a more flexible design of the nucleotide sequence to avoid hairpins, primer dimer structures and other inappropriate matches among the sequences which can hinder gene synthesis by polymerase chain reaction (PCR).
3. A total of 32 oligonucleotides (end concentration 250 nM) were mixed for the first PCR reaction to assemble them and built the designed gene sequence (30 cycles: 94° C for 15 s, 55°C for 30 s and 72°C for 45 s; Platinum ® Taq DNA Polymerase, Invitrogen, Carlsbad, CA).
4. A second PCR was used to amplify the full-length product using a forward and a reverse primer (30 cycles: 94°C for 15 s, 55°C for 30 s and 72°C for 45 s).
5. PCR products from this second run were purified by gel electrophoresis, digested with restriction enzymes (Promega, Madison, WI) and cloned into the polycloning site of the plasmid vector (EcoRI/XbaI digestion).
6. Bacteria were transformed with the ligated plasmids and clones were identified by sequence analysis (Applied Biosystem, CA, Sequencing core of UTSW).
7. An adenovirus E3 gene leader sequence and an endosomal targeting sequence were cloned up and down stream of the Aβ42 gene, respectively.
8. For the control immunizations corresponding plasmids were constructed. Plasmid pGal4/UAS-Luc consists of the same binary plasmid system as pGal4/UAS-Aβ42 trimer or monomer but without the E3 leader and endosomal targeting sequence, in which the transcription of the Luc

gene is driven by binding of the Gal4 transcription factor. In pCMV-Luc, transcription is driven by a CMV promoter.

DNA purification.

All plasmid DNAs were purified using a commercial plasmid maxi kit (Qiagen, Valencia, CA). The purity and concentration of DNA were measured by optical density reading at 260/280 nm and gel electrophoresis. Qiagen endotoxin-free DNA purification kit may be needed for electroporation vaccine.

DNA-Gold particle preparations (Advanced Protocol for clinic preparation).

1. In a 1.5 ml microfuge tube (Siliconized, Fisher brand #05-541-27), weigh 60mg gold microcarriers (Degussa Corporation Comgitm # and Batch # 33451 60021-05.
2. Wash twice with 100% alcohol, dry in 40°C.
3. Add 270 ug of p4u-Ab42 trimer (routinely 70 ug for mouse vaccine) and 54 ug of pCMVi-gal4, (routinely 14 ug) total DNA 324 ug (routinely 74 ug).
4. Add 100 µl of 0.05 M spermidine.
5. Vortex the gold and spermidine mixture for 10 seconds.
6. While vortexing the mixture at moderate rate speed vortexer, add 100 µl 2.5 M $CaCl_2$ dropwise to the mixture.
7. Allow the mixture to precipitate on ice for 15 minutes.
8. Spin the microcarrier solution in a microfuge 1 minute (3000 rpm) to pellet the gold.
9. Remove the supernatant and discard.
10. Wash the pellet three times with 1 ml of fresh 100% ethanol each time.
11. After the final ethanol wash, resuspend the pellet in 1.5 ml of the ethanol.
12. The suspension is now ready for tube preparation. Alternatively, the DNA/microcarrier suspensions can be stored for up to 2 months at -20°C. Prior to freezing, tighten the cap securely and put Parafilm® around the cap of the tube. After storage at -20°C, allow the particle suspension to come to room temperature prior to breaking the Parafilm seal.
13. Loading the DNA/Microcarrier Suspension into Gold-Coat Tubing Using the Tubing Prep Station.
14. Allow the microcarriers to settle for 3–5 minutes. Suck out the ethanol.
15. Flow nitrogen in 0.35–0.4 LPM of nitrogen to dry the Gold-Coat tubing.
16. Continue drying the Gold-Coat tubing while turning for 3–5 minutes.
17. Remove the tubing from the tubing support cylinder.
18. Cut into 0.5" cartridges put into a container.
19. Cap the container tightly, label, wrap with Parafilm, and store at -20°C.

20. The gold particle per cartridge (bullet) is about 1.5 mg gold with about 3.8 ug P4U-Ab42 trimer and 0.96 ug CMVi-Gal4 after freezing for 24 hours and thaw once. The DNA amount per bullet will further be tested after one week and one month storage in -20°C and P4U-Ab42 trimer should be in about 3.5 ug per bullet.

Figure 1 is a graph that shows the DNA binding to gold particles. The optimal ratio of DNA to the gold is 4.5 ug DNA (p4u-Ab42 trimer) with 1 mg gold. In this ratio, about 3.8 ug Ab42 trimer DNA can be bind to 1.5 mg gold per cartridge (Bullet) plus 20% CMVi-Gal4 DNA as additional.

Plasmid DNA sequence.

P4U-H3 (Abeta trimer).

Sequence: p4UK-H3 Range: 1 to 4600

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          >AseI    >SnaBI
            |       |
            | 10    |    20         30         40         50
    TAGTTATTAATTACGTAGGCTTAACTATGCGGCATCAGAGCAGATTGTAC
    ATCAATAATTAATGCATCCGAATTGATACGCCGTAGTCTCGTCTAACATG

                                  >SbfI
                                    |
                  >HindIII          >PstI
                     |                |
              60     |   70         80|        90        100
    TGAGAGTGCACCATAAGCTTGCATGCCTGCAGGTCGAAGCGGAGTACTGT
    ACTCTCACGTGGTATTCGAACGTACGGACGTCCAGCTTCGCCTCATGACA
            ___1 TO 527 OF P4U-AB42TRIMERAMP_______> 110
                     120       130       140         150

  CCTCCGAGCGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTTCGAGCGGA
  GGAGGCTCGCCTCATGACAGGAGGCTCGCCTCATGACAGGAAGCTCGCCT
  _________1 TO 527 OF P4U-AB42TRIMERAMP.SEQ__________>

                >HincII                     >XhoI
                   |                          |
                >AccI                       >TliI
                  ||                         |
               >SalI                       >PaeR7I
                 |||                         |
         160     |||170        180        190 |     200
  GTACTGTCCTCCGAGTCGACTCTAGAGGGTATATAATGGATCTCGAGATG
  CATGACAGGAGGCTCAGCTGAGATCTCCCATATATTACCTAGAGCTCTAC
  ________1 TO 527 OF P4U-AB42TRIMERAMP.SEQ__________>

                      >TspMI    >XhoI
                         |       |
                       >XmaI    >TliI
```

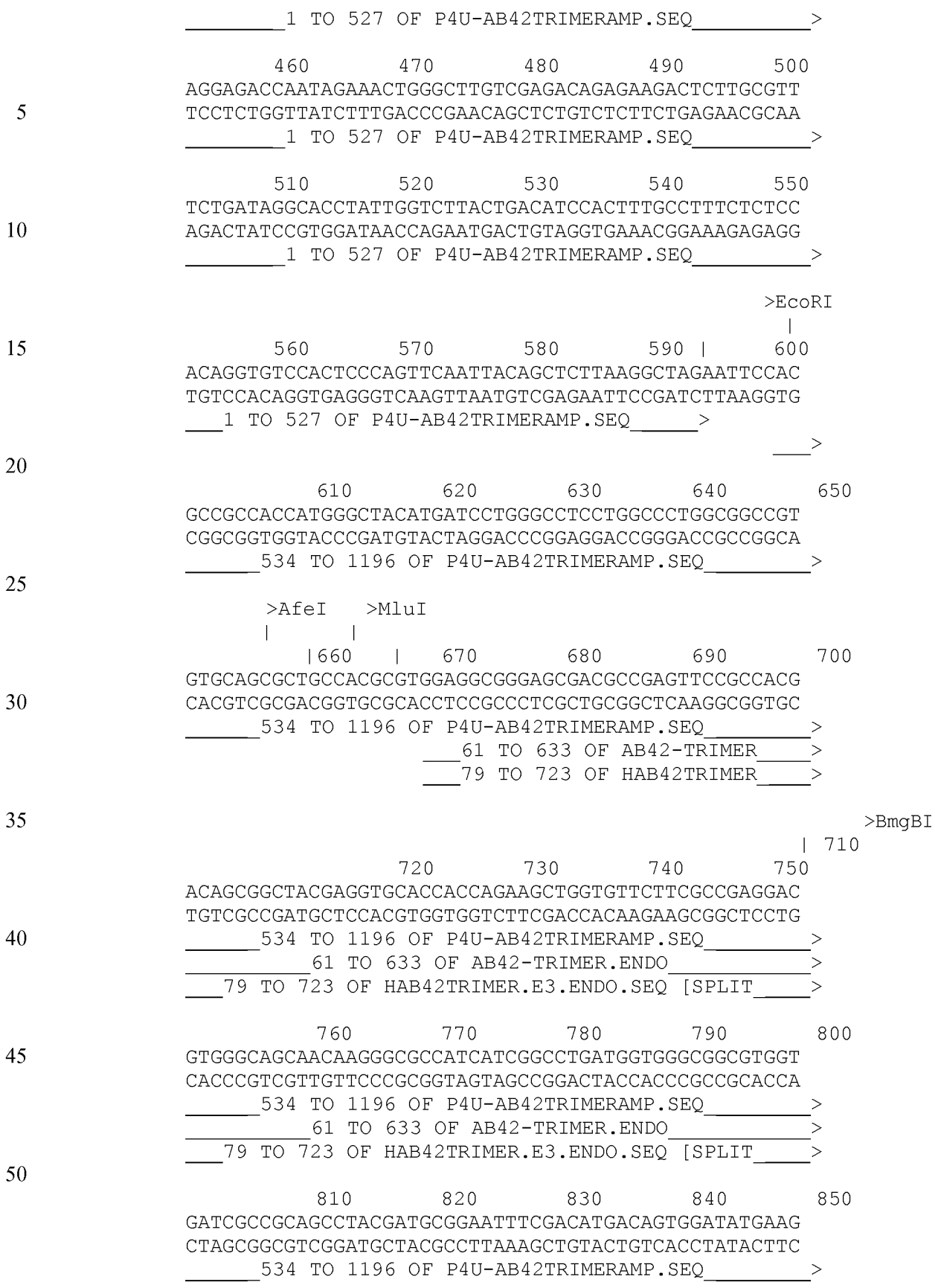
```
                          |     |
                >MluI     >BmtI    >PaeR7I
                  |          ||     |
          >SacI |  >NheI   |>SmaI |  >BglII
            |   |     |    ||  |   |   |
           210  |    220   ||  | 230   |   240       250
TACCGAGCTCTTACGCGTGCTAGCCCGGGCTCGAGATCTGGGCGGTAGGC
ATGGCTCGAGAATGCGCACGATCGGGCCCGAGCTCTAGACCCGCCATCCG
________1 TO 527 OF P4U-AB42TRIMERAMP.SEQ__________>

                                     >SacI
                                       |
          260       270       280 |     290       300
GTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAG
CACATGCCACCCTCCAGATATATTCGTCTCGAGCAAATCACTTGGCAGTC
________1 TO 527 OF P4U-AB42TRIMERAMP.SEQ__________>

                  >BmtI
                    |
           >NheI |
             |   |
          310|   |  320       330       340       350
ATCACTAGAAGCTAGCTTTATTGCGGTAGTTTATCACAGTTAAATTGCTA
TAGTGATCTTCGATCGAAATAACGCCATCAAATAGTGTCAATTTAACGAT
________1 TO 527 OF P4U-AB42TRIMERAMP.SEQ__________>

                                                    >PstI
                                                     |
          360       370       380       390    |  400
ACGCAGTCAGTGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGAAGT
TGCGTCAGTCACGAAGACTGTGTTGTCAGAGCTTGAATTCGACGTCTTCA
________1 TO 527 OF P4U-AB42TRIMERAMP.SEQ__________>

          410       420       430       440       450
TGGTCGTGAGGCACTGGGCAGGTAAGTATCAAGGTTACAAGACAGGTTTA
ACCAGCACTCCGTGACCCGTCCATTCATAGTTCCAATGTTCTGTCCAAAT
```


_____1 TO 527 OF P4U-AB42TRIMERAMP.SEQ_____>
460 470 480 490 500
AGGAGACCAATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGCGTT
TCCTCTGGTTATCTTTGACCCGAACAGCTCTGTCTCTTCTGAGAACGCAA
_____1 TO 527 OF P4U-AB42TRIMERAMP.SEQ_____>
510 520 530 540 550
TCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCC
AGACTATCCGTGGATAACCAGAATGACTGTAGGTGAAACGGAAAGAGAGG
_____1 TO 527 OF P4U-AB42TRIMERAMP.SEQ_____>
>EcoRI
|
560 570 580 590 | 600
ACAGGTGTCCACTCCCAGTTCAATTACAGCTCTTAAGGCTAGAATTCCAC
TGTCCACAGGTGAGGGTCAAGTTAATGTCGAGAATTCCGATCTTAAGGTG
___1 TO 527 OF P4U-AB42TRIMERAMP.SEQ_____>
___>
610 620 630 640 650
GCCGCCACCATGGGCTACATGATCCTGGGCCTCCTGGCCCTGGCGGCCGT
CGGCGGTGGTACCCGATGTACTAGGACCCGGAGGACCGGGACCGCCGGCA
_____534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_____>
>AfeI >MluI
| |
|660 | 670 680 690 700
GTGCAGCGCTGCCACGCGTGGAGGCGGGAGCGACGCCGAGTTCCGCCACG
CACGTCGCGACGGTGCGCACCTCCGCCCTCGCTGCGGCTCAAGGCGGTGC
_____534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_____>
___61 TO 633 OF AB42-TRIMER____>
___79 TO 723 OF HAB42TRIMER____>
>BmgBI
| 710
720 730 740 750
ACAGCGGCTACGAGGTGCACCACCAGAAGCTGGTGTTCTTCGCCGAGGAC
TGTCGCCGATGCTCCACGTGGTGGTCTTCGACCACAAGAAGCGGCTCCTG
_____534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_____>
_____61 TO 633 OF AB42-TRIMER.ENDO_____>
___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT____>
760 770 780 790 800
GTGGGCAGCAACAAGGGCGCCATCATCGGCCTGATGGTGGGCGGCGTGGT
CACCCGTCGTTGTTCCCGCGGTAGTAGCCGGACTACCACCCGCCGCACCA
_____534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_____>
_____61 TO 633 OF AB42-TRIMER.ENDO_____>
___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT____>
810 820 830 840 850
GATCGCCGCAGCCTACGATGCGGAATTTCGACATGACAGTGGATATGAAG
CTAGCGGCGTCGGATGCTACGCCTTAAAGCTGTACTGTCACCTATACTTC
_____534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_____>

```
          ___________61 TO 633 OF AB42-TRIMER.ENDO_____________>
          ___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT_____>

                    860       870       880       890       900
          TACATCACCAAAAACTCGTATTTTTCGCGGAAGATGTAGGAAGCAACAAG
          ATGTAGTGGTTTTTGAGCATAAAAAGCGCCTTCTACATCCTTCGTTGTTC
          _______534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_________>
          ___________61 TO 633 OF AB42-TRIMER.ENDO_____________>
          ___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT_____>

                    910       920       930       940       950
          GGAGCAATCATAGGACTAATGGTAGGAGGGGTAGTCATAGCAGCGGCTTA
          CCTCGTTAGTATCCTGATTACCATCCTCCCCATCAGTATCGTCGCCGAAT
          _______534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_________>
          ___________61 TO 633 OF AB42-TRIMER.ENDO_____________>
          ___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT_____>

                    960       970       980       990      1000
          TGATGCTGAATTTCGTCATGATTCGGGTTATGAAGTTCATCATCAAAAAT
          ACTACGACTTAAAGCAGTACTAAGCCCAATACTTCAAGTAGTAGTTTTTA
          _______534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_________>
          ___________61 TO 633 OF AB42-TRIMER.ENDO_____________>
          ___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT_____>

                   1010      1020      1030      1040      1050
          TAGTGTTTTTCGCTGAAGATGTTGGTTCTAATAAAGGAGCTATTATAGGT
          ATCACAAAAAGCGACTTCTACAACCAAGATTATTTCCTCGATAATATCCA
          _______534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_________>
          ___________61 TO 633 OF AB42-TRIMER.ENDO_____________>
          ___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT_____>

                                                    >BglII
                                                      |
                   1060      1070      1080      1090      1100
          TTAATGGTTGGGGGTGTTGTTATTGCTGGTGGCGGTTCGAGATCTATCCA
          AATTACCAACCCCCACAACAATAACGACCACCGCCAAGCTCTAGATAGGT
          _______534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_________>
          ___________61 TO 633 OF AB42-TRIMER.ENDO_____________>
          ___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT_____>

                   1110      1120      1130      1140      1150
          GACCGTCAAGGTGAGCGTGAGCGCCGCCACCCTGGGCCTGGGCTTCATCA
          CTGGCAGTTCCACTCGCACTCGCGGCGGTGGGACCCGGACCCGAAGTAGT
          _______534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_________>
          ___________61 TO 633 OF AB42-TRIMER.ENDO_____________>
          ___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT_____>

                   1160      1170      1180      1190      1200
          TCTTCTGCGTGGGGTTCTTCCGGTGGCGCAAGAGCCACTCCTCCAGCTAC
          AGAAGACGCACCCCAAGAAGGCCACCGCGTTCTCGGTGAGGAGGTCGATG
          _______534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_________>
          ___________61 TO 633 OF AB42-TRIMER.ENDO_____________>
          ___79 TO 723 OF HAB42TRIMER.E3.ENDO.SEQ [SPLIT_____>
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                                                        >HindIII
                                                           |
      1210      1220      1230      1240   |    1250
ACCCCCCTCTCCGGCTCCACCTATCCCGAGGGGCGCCACTAGAAGCTTTC
TGGGGGGAGAGGCCGAGGTGGATAGGGCTCCCCGCGGTGATCTTCGAAAG
______534 TO 1196 OF P4U-AB42TRIMERAMP.SEQ_________>
______61 TO 633 OF AB42-TRIMER.ENDO_______>
___79 TO 723 OF HAB42TRIMER.E3.ENDO.SE_  _>

                   >NotI
                     |
      1260      1270      1280      1290      1300
TAGTTCTAGAGCACTGGCGGCCGCGACTCTAGATCATAATCAGCCATACC
ATCAAGATCTCGTGACCGCCGGCGCTGAGATCTAGTATTAGTCGGTATGG
__________>

      1310      1320      1330      1340      1350
ACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCT
TGTAAACATCTCCAAAATGAACGAAATTTTTTGGAGGGTGTGGAGGGGGA

                       >BsmI       >HpaI
                         |           |
                      >MfeI        >HincII
                        ||            |
      1360      1370    || 1380      1390      1400
GAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTG
CTTGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAATAAC

        >PsiI
      |
      1410      1420      1430      1440      1450
CAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAAT
GTCGAATATTACCAATGTTTATTTCGTTATCGTAGTGTTTAAAGTGTTTA

                   >BsmI
                     |
      1460      1470      1480      1490      1500
AAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAA
TTTCGTAAAAAAAGTGACGTAAGATCAACACCAAACAGGTTTGAGTAGTT

                                    >SspI
                                      |
      1510      1520      1530 |    1540      1550
        TGTATCTTAAGGCGTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCG
        ACATAGAATTCCGCATTTAACATTCGCAATTATAAAACAATTTTAAGCGC

      1560      1570      1580      1590      1600
TTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGG
AATTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTAGCC

             >PsiI
               |
      1610 |    1620      1630      1640      1650
CAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTG
```

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                GTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAACTCACAAC

                     1660      1670      1680      1690      1700
                TTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTC
                AAGGTCAAACCTTGTTCTCAGGTGATAATTTCTTGCACCTGAGGTTGCAG

                     1710      1720      1730      1740      1750
                AAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATC
                TTTCCCGCTTTTTGGCAGATAGTCCCGCTACCGGGTGATGCACTTGGTAG

                     1760      1770      1780      1790      1800
                ACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGA
                TGGGATTAGTTCAAAAAACCCCAGCTCCACGGCATTTCGTGATTTAGCCT

                     1810      1820      1830      1840      1850
                ACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAAC
                TGGGATTTCCCTCGGGGGCTAAATCTCGAACTGCCCCTTTCGGCCGCTTG

                     1860      1870      1880      1890      1900
                GTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCT
                CACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGCGATCCCGCGA

                     1910      1920      1930      1940      1950
                GGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTA
                CCGTTCACATCGCCAGTGCGACGCGCATTGGTGGTGTGGGCGGCGCGAAT

                     1960      1970      1980      1990      2000
                ATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGC
                TACGCGGCGATGTCCCGCGCAGTCCACCGTGAAAAGCCCCTTTACACGCG

                     2010      2020      2030      2040      2050
                GGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCT
                CCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGA

                                              >SspI
                                                |
                     2060      2070      2080    | 2090      2100
                CATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGA
                GTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCT

>Bsu36I             >PvuII
       |                 |
          |  2110      2120 |    2130      2140      2150
                GTCCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTG
                CAGGACTCCGCCTTTCTTGGTCGACACCTTACACACAGTCAATCCCACAC

                     2160      2170      2180      2190      2200
                GAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTC
                CTTTCAGGGGTCCGAGGGGTCGTCCGTCTTCATACGTTTCGTACGTAGAG

                 >SexAI
                   |
                     2210  |   2220      2230      2240      2250
                AATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAG
```

```
                TTAATCAGTCGTTGGTCCACACCTTTCAGGGGTCCGAGGGGTCGTCCGTC

                     2260      2270      2280      2290      2300
                AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC
                TTCATACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTATCAGGGCGGGG

                     2310      2320      2330      2340      2350
                TAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCG
                ATTGAGGCGGGTAGGGCGGGGATTGAGGCGGGTCAAGGCGGGTAAGAGGC

                     2360      2370      2380      2390      2400
                CCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTC
                GGGGTACCGACTGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAG

                                                        >AvrII
                                                          |
                                                       >StuI
                                                    || 2410
                          2420      2430      2440    ||2450
                GGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAG
                CCGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATC

                       >ClaI
                         |
                       >BspDI
                         |
                     2460   |  2470      2480      2490      2500
                GCTTTTGCAAAGATCGATCAAGAGACAGGATGAGGATCGTTTCGCATGAT
                CGAAAACGTTTCTAGCTAGTTCTCTGTCCTACTCCTAGCAAAGCGTACTA

                     2510      2520      2530      2540      2550
                TGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGC
                ACTTGTTCTACCTAACGTGCGTCCAAGAGGCCGGCGAACCCACCTCTCCG

                     2560      2570      2580      2590      2600
                  TATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCC
                ATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGG

                     2610      2620      2630      2640      2650
                GTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGA
                  CACAAGGCCGACAGTCGCGTCCCCGCGGGCCAAGAAAAACAGTTCTGGCT

                     2660      2670      2680      2690      2700
                CCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCGT
                GGACAGGCCACGGGACTTACTTGACGTTCTGCTCCGTCGCGCCGATAGCA

                                        >PvuII
                                          |
              >MscI                  >FspI |
                |                       |   |
                |2710      2720      |2730      2740      2750
          GGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACT
          CCGACCGGTGCTGCCCGCAAGGAACGCGTCGACACGAGCTGCAACAGTGA
```

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          2760      2770      2780      2790      2800
GAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCT
CTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGA

          2810      2820      2830      2840      2850
CCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATG
GGACAGTAGAGTGGAACGAGGACGGCTCTTTCATAGGTAGTACCGACTAC

          2860      2870      2880      2890      2900
CAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCAC
GTTACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTG

          2910      2920      2930      2940      2950
CAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCT
GTTCGCTTTGTAGCGTAGCTCGCTCGTGCATGAGCCTACCTTCGGCCAGA

          2960      2970      2980      2990      3000
TGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCG
ACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGC

          3010      3020      3030      3040      3050
AACTGTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTC
TTGACAAGCGGTCCGAGTTCCGCTCGTACGGGCTGCCGCTCCTAGAGCAG

          3060      3070      3080      3090      3100
GTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCG
CACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGC

                                                >RsrII
                                                  |
                                                >AvaII
                                                  |
          3110      3120      3130      3140      3150
CTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATC
GAAAAGACCTAAGTAGCTGACACCGGCCGACCCACACCGCCTGGCGATAG

          3160      3170      3180      3190      3200
AGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAA
TCCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGCCGCTT

          3210      3220      3230      3240      3250
TGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCA
ACCCGACTGGCGAAGGAGCACGAAATGCCATAGCGGCGAGGGCTAAGCGT

          3260      3270      3280      3290      3300
GCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCT
CGCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGA

  >BstBI
  |
         |3310      3320      3330      3340      3350
GGGGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATT
CCCCAAGCTTTACTGGCTGGTTCGCTGCGGGTTGGACGGTAGTGCTCTAA
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      3360       3370       3380       3390       3400
TCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTC
AGCTAAGGTGGCGGCGGAAGATACTTTCCAACCCGAAGCCTTAGCAAAAG

      3410       3420       3430       3440       3450
CGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTT
GCCCTGCGGCCGACCTACTAGGAGGTCGCGCCCCTAGAGTACGACCTCAA

    >AvrII
      |
      3460|      3470       3480       3490       3500
CTTCGCCCACCCTAGGGGGAGGCTAACTGAAACACGGAAGGAGACAATAC
GAAGCGGGTGGGATCCCCCTCCGATTGACTTTGTGCCTTCCTCTGTTATG

      3510       3520       3530       3540       3550
CGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCAC
GCCTTCCTTGGGCGCGATACTGCCGTTATTTTTCTGTCTTATTTTGCGTG

                                >AvaII
                                  |
      3560       3570       3580     | 3590       3600
GGTGTTGGGTCGTTTGTTCATAAACGCGGGGTTCGGTCCCAGGGCTGGCA
CCACAACCCAGCAAACAAGTATTTGCGCCCCAAGCCAGGGTCCCGACCGT

      3610       3620       3630       3640       3650
CTCTGTCGATACCCCACCGAGACCCCATTGGGGCCAATACGCCCGCGTTT
GAGACAGCTATGGGGTGGCTCTGGGGTAACCCCGGTTATGCGGGCGCAAA

      3660       3670       3680       3690       3700
CTTCCTTTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGGCCCAGGGCTC
GAAGGAAAAGGGGTGGGGTGGGGGGTTCAAGCCCACTTCCGGGTCCCGAG

                >EcoO109I       >Bsu36I
                    |               |
      3710       3720  |     3730      |3740       3750
GCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGCCTCAGGTTACTCATA
CGTCGGTTGCAGCCCCGCCGTCCGGGACGGTATCGGAGTCCAATGAGTAT

      3760       3770       3780       3790       3800
TATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGG
   ATATGAAATCTAACTAAATTTTGAAGTAAAAATTAAATTTTCCTAGATCC

      3810       3820       3830       3840       3850
TGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTT
ACTTCTAGGAAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAA

      3860       3870       3880       3890       3900
TCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTG
AGCAAGGTGACTCGCAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAAC

      3910       3920       3930       3940       3950
AGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCAC
TCTAGGAAAAAAAGACGCGCATTAGACGACGAACGTTTGTTTTTTTGGTG
```

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      3960       3970       3980       3990       4000
CGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTT
GCGATGGTCGCCACCAAACAAACGGCCTAGTTCTCGATGGTTGAGAAAAA

      4010       4020       4030       4040       4050
CCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCT
GGCTTCCATTGACCGAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGA

      4060       4070       4080       4090       4100
AGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTA
TCACATCGGCATCAATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGAT

      4110       4120       4130       4140       4150
CATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGAT
GTATGGAGCGAGACGATTAGGACAATGGTCACCGACGACGGTCACCGCTA

      4160       4170       4180       4190       4200
AAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGC
TTCAGCACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCG

      4210       4220       4230       4240       4250
GCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGC
CGTCGCCAGCCCGACTTGCCCCCCAAGCACGTGTGTCGGGTCGAACCTCG

      4260       4270       4280       4290       4300
GAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGC
CTTGCTGGATGTGGCTTGACTCTATGGATGTCGCACTCGATACTCTTTCG

      4310       4320       4330       4340       4350
GCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAG
CGGTGCGAAGGGCTTCCCTCTTTCCGCCTGTCCATAGGCCATTCGCCGTC

      4360       4370       4380       4390       4400
GGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGT
CCAGCCTTGTCCTCTCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCA

      4410       4420       4430       4440       4450
ATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTT
TAGAAATATCAGGACAGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAA

      4460       4470       4480       4490       4500
TTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGC
AACACTACGAGCAGTCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCG

                                              >PciI
                                                |
      4510       4520       4530       4540  |    4550
GGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCT
CCGGAAAAATGCCAAGGACCGGAAAACGACCGGAAAACGAGTGTACAAGA

      4560       4570       4580       4590       4600
TTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT
AAGGACGCAATAGGGGACTAAGACACCTATTGGCATAATGGCGGTACGTA
```

(SED ID NO:1)

Pcmv-Gal4.

```
                    >NruI                 >MluI                  >SpeI
                      |                     |                     | 10
                                  20        30        40        50
                   GACTCTTCGCGATGTACGGGCCAGATATACGCGTTGACATTGATTATTGA
                   CTGAGAAGCGCTACATGCCCGGTCTATATGCGCAACTGTAACTAATAACT

                     >AseI
                     |
                      |60        70        80        90       100
                   CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
                   GATCAATAATTATCATTAGTTAATGCCCCAGTAATCAAGTATCGGGTATA

                      110       120       130       140       150
                   ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACC
                   TACCTCAAGGCGCAATGTATTGAATGCCATTTACCGGGCGGACCGACTGG

                      160       170       180       190       200
                   GCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAG
                   CGGGTTGCTGGGGGCGGGTAACTGCAGTTATTACTGCATACAAGGGTATC

                      210       220       230       240       250
                   TAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGG
                   ATTGCGGTTATCCCTGAAAGGTAACTGCAGTTACCCACCTGATAAATGCC

                                                >NdeI
                                                  |
                      260       270       280     |  290       300
                   TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCC
                   ATTTGACGGGTGAACCGTCATGTAGTTCACATAGTATACGGTTCATGCGG

                      310       320       330       340       350
                   CCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
                   GGGATAACTGCAGTTACTGCCATTTACCGGGCGGACCGTAATACGGGTCA

                                                          >SnaBI
                                                            |
                      360       370       380       390|      400
                   ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTC
                   TGTACTGGAATACCCTGAAAGGATGAACCGTCATGTAGATGCATAATCAG

                     >NcoI
                       |
                     >BtgI
                       |
                      410|      420       430       440       450
                   ATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGG
                   TAGCGATAATGGTACCACTACGCCAAAACCGTCATGTAGTTACCCGCACC

                      460       470       480       490       500
                   ATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCA
                   TATCGCCAAACTGAGTGCCCCTAAAGGTTCAGAGGTGGGGTAACTGCAGT
```

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                 510       520       530       540       550
          ATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGT
          TACCCTCAAACAAAACCGTGGTTTTAGTTGCCCTGAAAGGTTTTACAGCA

                 560       570       580       590       600
          AACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGA
          TTGTTGAGGCGGGGTAACTGCGTTTACCCGCCATCCGCACATGCCACCCT

                      >SacI
                        |
                 610       620       630       640       650
          GGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACT
          CCAGATATATTCGTCTCGAGAGACCGATTGATCTCTTGGGTGACGAATGA

                                                          >BmtI
                                                            |
                    >AseI                             >NheI |
                      |                                 |   |
                   660  |    670       680       690     | 700
GGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAG
CCGAATAGCTTTAATTATGCTGAGTGATATCCCTCTGGGTTCGACCGATC

                              >KpnI
                                |
          >AflII    >Acc65I|      >BamHI
             |         |    |        |
   >PmeI   |>HindIII|    |    >SacI    >SpeI
      |    |  |      |    |      | |      |
      |  710 |     720 |     730      | 740        750

CGTTTAAACTTAAGCTTGGTACCGAGCTCGGATCCACTAGTCCAGTGTGG
GCAAATTTGAATTCGAACCATGGCTCGAGCCTAGGTGATCAGGTCACACC

>EcoRI
  |
  |    760       770       780       790       800
TGGAATTCCACGCCGCCACCATGAAGCTACTGTCTTCTATCGAACAAGCA
ACCTTAAGGTGCGGCGGTGGTACTTCGATGACAGAAGATAGCTTGTTCGT
        __________1 TO 2658 OF GAL4.DNA___________>

       810       820       830       840       850
TGCGATATTTGCCGACTTAAAAAGCTCAAGTGCTCCAAAGAAAAACCGAA
ACGCTATAAACGGCTGAATTTTTCGAGTTCACGAGGTTTCTTTTTGGCTT
_______1 TO 2658 OF GAL4.DNA_____________________>

       860       870       880       890       900
GTGCGCCAAGTGTCTGAAGAACAACTGGGAGTGTCGCTACTCTCCCAAAA
CACGCGGTTCACAGACTTCTTGTTGACCCTCACAGCGATGAGAGGGTTTT
_______1 TO 2658 OF GAL4.DNA_____________________>

       910       920       930       940       950
CCAAAAGGTCTCCGCTGACTAGGGCACATCTGACAGAAGTGGAATCAAGG
GGTTTTCCAGAGGCGACTGATCCCGTGTAGACTGTCTTCACCTTAGTTCC
_____________1 TO 2658 OF GAL4.DNA_______________>
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```
                                                >AvaI
                                                    |
                                                >BsoBI
                                                    |
                                               >PaeR7I
                                                    |
                                                >TliI
                                                    |
                                                >XhoI
                                                    |
            960       970       980       990       1000
CTAGAAAGACTGGAACAGCTATTTCTACTGATTTTTCCTCGAGAAGACCT
GATCTTTCTGACCTTGTCGATAAAGATGACTAAAAAGGAGCTCTTCTGGA
_____________1 TO 2658 OF GAL4.DNA_____________>

                                                          >HpaI
                                                       | 1010
                 1020      1030      1040      1050
TGACATGATTTTGAAAATGGATTCTTTACAGGATATAAAAGCATTGTTAA
ACTGTACTAAAACTTTTACCTAAGAAATGTCCTATATTTTCGTAACAATT
_____________1 TO 2658 OF GAL4.DNA_____________>

        >BsrGI
           |
      1060|     1070      1080      1090      1100
CAGGATTATTTGTACAAGATAATGTGAATAAAGATGCCGTCACAGATAGA
GTCCTAATAAACATGTTCTATTACACTTATTTCTACGGCAGTGTCTATCT
______1 TO 2658 OF GAL4.DNA_____________________>

      1110      1120      1130      1140      1150
TTGGCTTCAGTGGAGACTGATATGCCTCTAACATTGAGACAGCATAGAAT
AACCGAAGTCACCTCTGACTATACGGAGATTGTAACTCTGTCGTATCTTA
______1 TO 2658 OF GAL4.DNA_____________________>

      1160      1170      1180      1190      1200
AAGTGCGACATCATCATCGGAAGAGAGTAGTAACAAAGGTCAAAGACAGT
TTCACGCTGTAGTAGTAGCCTTCTCTCATCATTGTTTCCAGTTTCTGTCA
_____________1 TO 2658 OF GAL4.DNA_____________>

      >ClaI
         |
      >BspDI
         |
      1210      1220      1230      1240      1250
TGACTGTATCGATTGACTCGGCAGCTCATCATGATAACTCCACAATTCCG
ACTGACATAGCTAACTGAGCCGTCGAGTAGTACTATTGAGGTGTTAAGGC
______1 TO 2658 OF GAL4.DNA_____________________>

      1260      1270      1280      1290      1300
TTGGATTTTATGCCCAGGGATGCTCTTCATGGATTTGATTGGTCTGAAGA
AACCTAAAATACGGGTCCCTACGAGAAGTACCTAAACTAACCAGACTTCT
_____________1 TO 2658 OF GAL4.DNA_____________>

   >PciI
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     |
        |1310      1320      1330      1340      1350
GGATGACATGTCGGATGGCTTGCCCTTCCTGAAAACGGACCCCAACAATA
CCTACTGTACAGCCTACCGAACGGGAAGGACTTTTGCCTGGGGTTGTTAT
______1 TO 2658 OF GAL4.DNA_____________________>

          1360      1370      1380      1390      1400
ATGGGTTCTTTGGCGACGGTTCTCTCTTATGTATTCTTCGATCTATTGGC
TACCCAAGAAACCGCTGCCAAGAGAGAATACATAAGAAGCTAGATAACCG
_____________1 TO 2658 OF GAL4.DNA_______________>

                                  >HpaI
                                    |
                                >AclI |
                                  |   |
          1410      1420      1430  |   1440      1450
TTTAAACCGGAAAATTACACGAACTCTAACGTTAACAGGCTCCCGACCAT
AAATTTGGCCTTTTAATGTGCTTGAGATTGCAATTGTCCGAGGGCTGGTA
_____________1 TO 2658 OF GAL4.DNA_______________>

                            >XbaI
                              |
          1460      1470     |1480      1490      1500
GATTACGGATAGATACACGTTGGCTTCTAGATCCACAACATCCCGTTTAC
CTAATGCCTATCTATGTGCAACCGAAGATCTAGGTGTTGTAGGGCAAATG
_____________1 TO 2658 OF GAL4.DNA_______________>

                                                >ApaLI
                                                  |
          1510      1520      1530      1540 |    1550
TTCAAAGTTATCTCAATAATTTTCACCCCTACTGCCCTATCGTGCACTCA
AAGTTTCAATAGAGTTATTAAAAGTGGGGATGACGGGATAGCACGTGAGT
______1 TO 2658 OF GAL4.DNA_____________________>

          1560      1570      1580      1590      1600
CCGACGCTAATGATGTTGTATAATAACCAGATTGAAATCGCGTCGAAGGA
GGCTGCGATTACTACAACATATTATTGGTCTAACTTTAGCGCAGCTTCCT
______1 TO 2658 OF GAL4.DNA_____________________>

          1610      1620      1630      1640      1650
TCAATGGCAAATCCTTTTTAACTGCATATTAGCCATTGGAGCCTGGTGTA
AGTTACCGTTTAGGAAAAATTGACGTATAATCGGTAACCTCGGACCACAT
______1 TO 2658 OF GAL4.DNA_____________________>

          1660      1670      1680      1690      1700
TAGAGGGGGAATCTACTGATATAGATGTTTTTTACTATCAAAATGCTAAA
ATCTCCCCCTTAGATGACTATATCTACAAAAAATGATAGTTTTACGATTT
______1 TO 2658 OF GAL4.DNA_____________________>

          1710      1720      1730      1740      1750
TCTCATTTGACGAGCAAGGTCTTCGAGTCAGGTTCCATAATTTTGGTGAC
AGAGTAAACTGCTCGTTCCAGAAGCTCAGTCCAAGGTATTAAAACCACTG
_____________1 TO 2658 OF GAL4.DNA_______________>
```

```
                    >NruI
                      |
      1760      1770      1780      1790      1800
AGCCCTACATCTTCTGTCGCGATATACACAGTGGAGGCAGAAAACAAATA
TCGGGATGTAGAAGACAGCGCTATATGTGTCACCTCCGTCTTTTGTTTAT
______1 TO 2658 OF GAL4.DNA_____________________>

      1810      1820      1830      1840      1850
CTAGCTATAATTTTCACAGCTTTTCCATAAGAATGGCCATATCATTGGGC
GATCGATATTAAAAGTGTCGAAAAGGTATTCTTACCGGTATAGTAACCCG
_____________1 TO 2658 OF GAL4.DNA_______________>

      >PpuMI                        >BsmI
           |                              |
      1860      1870      1880      1890      1900
TTGAATAGGGACCTCCCCTCGTCCTTCAGTGATAGCAGCATTCTGGAACA
AACTTATCCCTGGAGGGGAGCAGGAAGTCACTATCGTCGTAAGACCTTGT
_____________1 TO 2658 OF GAL4.DNA_______________>

                          >AccI            >MfeI
                            |                |
      1910      1920    | 1930      1940|     1950
AAGACGCCGAATTTGGTGGTCTGTCTACTCTTGGGAGATCCAATTGTCCC
TTCTGCGGCTTAAACCACCAGACAGATGAGAACCCTCTAGGTTAACAGGG
______1 TO 2658 OF GAL4.DNA_____________________>

      1960      1970      1980      1990      2000
TGCTTTATGGTCGATCCATCCAGCTTTCTCAGAATACAATCTCCTTCCCT
ACGAAATACCAGCTAGGTAGGTCGAAAGAGTCTTATGTTAGAGGAAGGGA
_____________1 TO 2658 OF GAL4.DNA_______________>

        >AccI
          |
       >SalI                      >PpuMI
      ||                           |
      2010      2020      2030    |  2040      2050
TCTTCTGTCGACGATGTGCAGCGTACCACAACAGGTCCCACCATATATCA
AGAAGACAGCTGCTACACGTCGCATGGTGTTGTCCAGGGTGGTATATAGT
______1 TO 2658 OF GAL4.DNA_____________________>

      2060      2070      2080      2090      2100
TGGCATCATTGAAACAGCAAGGCTCTTACAAGTTTTCACAAAAATCTATG
ACCGTAGTAACTTTGTCGTTCCGAGAATGTTCAAAAGTGTTTTTAGATAC
_____________1 TO 2658 OF GAL4.DNA_______________>

                        >PstI
                          |
      2110      2120  |   2130      2140      2150
AACTAGACAAAACAGTAACTGCAGAAAAAAGTCCTATATGTGCAAAAAAA
TTGATCTGTTTTGTCATTGACGTCTTTTTTCAGGATATACACGTTTTTTT
______1 TO 2658 OF GAL4.DNA_____________________>

      2160      2170      2180      2190      2200
TGCTTGATGATTTGTAATGAGATTGAGGAGGTTTCGAGACAGGCACCAAA
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ACGAACTACTAAACATTACTCTAACTCCTCCAAAGCTCTGTCCGTGGTTT
______1 TO 2658 OF GAL4.DNA_____________________>

          2210      2220      2230      2240      2250
GTTTTTACAAATGGATATTTCCACCACCGCTCTAACCAATTTGTTGAAGG
CAAAAATGTTTACCTATAAAGGTGGTGGCGAGATTGGTTAAACAACTTCC
_____________1 TO 2658 OF GAL4.DNA_______________>

                              >BstBI
                                |
          2260      2270      2280      2290      2300
AACACCCTTGGCTATCCTTTACAAGATTCGAACTGAAGTGGAAACAGTTG
TTGTGGGAACCGATAGGAAATGTTCTAAGCTTGACTTCACCTTTGTCAAC
______1 TO 2658 OF GAL4.DNA_____________________>

          2310      2320      2330      2340      2350
TCTCTTATCATTTATGTATTAAGAGATTTTTTCACTAATTTTACCCAGAA
AGAGAATAGTAAATACATAATTCTCTAAAAAAGTGATTAAAATGGGTCTT
______1 TO 2658 OF GAL4.DNA_____________________>

          2360      2370      2380      2390      2400
AAAGTCACAACTAGAACAGGATCAAAATGATCATCAAAGTTATGAAGTTA
TTTCAGTGTTGATCTTGTCCTAGTTTTACTAGTAGTTTCAATACTTCAAT
______1 TO 2658 OF GAL4.DNA_____________________>

          2410      2420      2430      2440      2450
AACGATGCTCCATCATGTTAAGCGATGCAGCACAAAGAACTGTTATGTCT
TTGCTACGAGGTAGTACAATTCGCTACGTCGTGTTTCTTGACAATACAGA
______1 TO 2658 OF GAL4.DNA_____________________>

          2460      2470      2480      2490      2500
GTAAGTAGCTATATGGACAATCATAATGTCACCCCATATTTTGCCTGGAA
CATTCATCGATATACCTGTTAGTATTACAGTGGGGTATAAAACGGACCTT
______1 TO 2658 OF GAL4.DNA_____________________>

          2510      2520      2530      2540      2550
TTGTTCTTATTACTTGTTCAATGCAGTCCTAGTACCCATAAAGACTCTAC
AACAAGAATAATGAACAAGTTACGTCAGGATCATGGGTATTTCTGAGATG
_____________1 TO 2658 OF GAL4.DNA_______________>

                          >BsmI
                            |
          2560      2570    |   2580      2590      2600
TCTCAAACTCAAAATCGAATGCTGAGAATAACGAGACCGCACAATTATTA
AGAGTTTGAGTTTTAGCTTACGACTCTTATTGCTCTGGCGTGTTAATAAT
______1 TO 2658 OF GAL4.DNA_____________________>

          2610      2620      2630      2640      2650
CAACAAATTAACACTGTTCTGATGCTATTAAAAAAACTGGCCACTTTTAA
GTTGTTTAATTGTGACAAGACTACGATAATTTTTTTGACCGGTGAAAATT
_____________1 TO 2658 OF GAL4.DNA_______________>

                                >ScaI
                                  |
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      2660      2670      2680      2690      2700
AATCCAGACTTGTGAAAAATACATTCAAGTACTGGAAGAGGTATGTGCGC
TTAGGTCTGAACACTTTTTATGTAAGTTCATGACCTTCTCCATACACGCG
_____________1 TO 2658 OF GAL4.DNA_____________>

                                                  >PsiI
                                                | 2710
                  2720      2730      2740     |2750
CGTTTCTGTTATCACAGTGTGCAATCCCATTACCGCATATCAGTTATAAC
GCAAAGACAATAGTGTCACACGTTAGGGTAATGGCGTATAGTCAATATTG
_____________1 TO 2658 OF GAL4.DNA_____________>

                            >SspI
                              |
      2760      2770      2780      2790      2800
AATAGTAATGGTAGCGCCATTAAAAATATTGTCGGTTCTGCAACTATCGC
TTATCATTACCATCGCGGTAATTTTTATAACAGCCAAGACGTTGATAGCG
_____________1 TO 2658 OF GAL4.DNA_____________>

                 >BspEI
                   |
      2810     |2820      2830      2840      2850
CCAATACCCTACTCTTCCGGAGGAAAATGTCAACAATATCAGTGTTAAAT
GGTTATGGGATGAGAAGGCCTCCTTTTACAGTTGTTATAGTCACAATTTA
______1 TO 2658 OF GAL4.DNA_____________________>

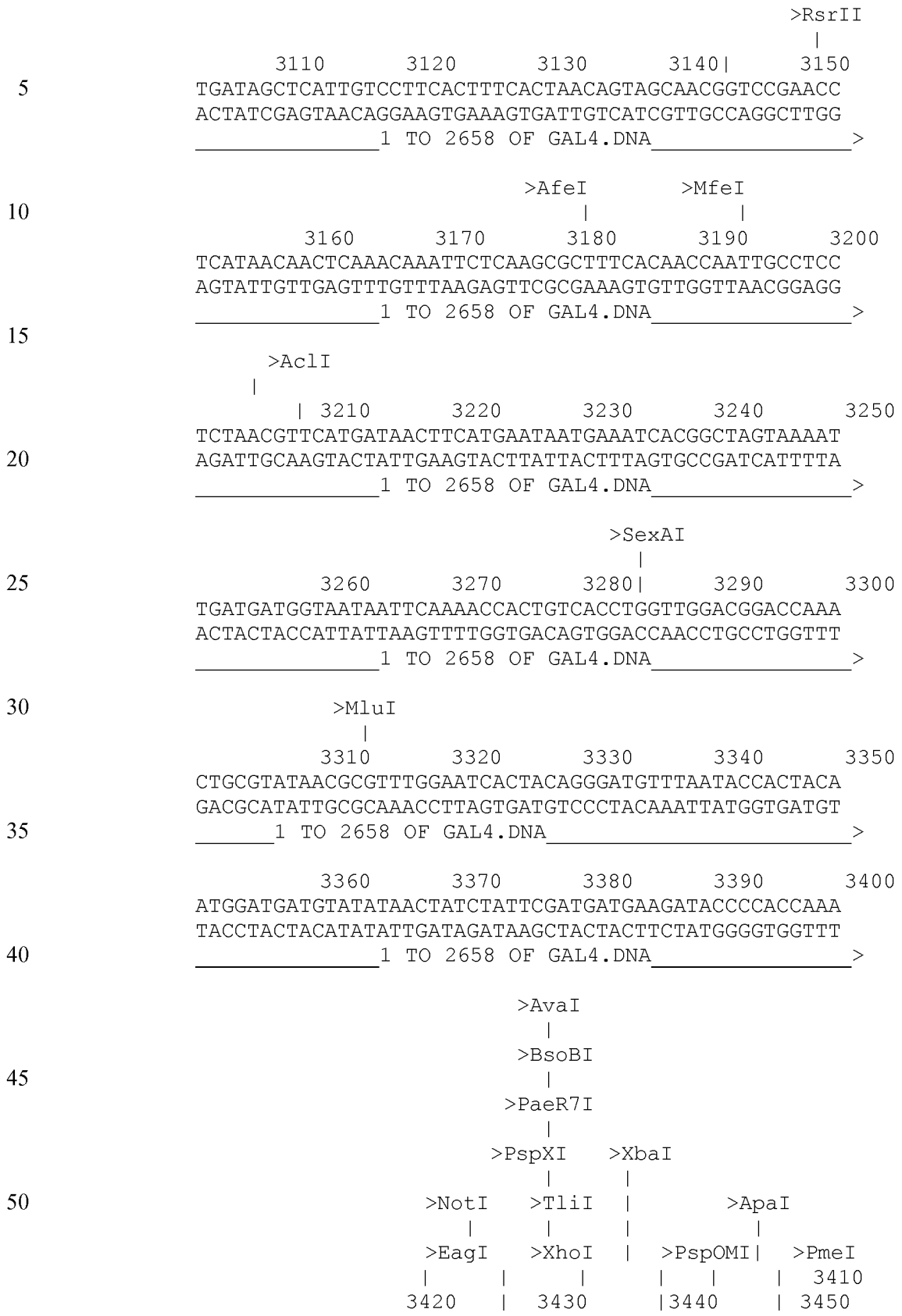
      2860      2870      2880      2890      2900
ATGTTTCTCCTGGCTCAGTAGGGCCTTCACCTGTGCCATTGAAATCAGGA
TACAAAGAGGACCGAGTCATCCCGGAAGTGGACACGGTAACTTTAGTCCT
______1 TO 2658 OF GAL4.DNA_____________________>

      2910      2920      2930      2940      2950
GCAAGTTTCAGTGATCTAGTCAAGCTGTTATCTAACCGTCCACCCTCTCG
CGTTCAAAGTCACTAGATCAGTTCGACAATAGATTGGCAGGTGGGAGAGC
______1 TO 2658 OF GAL4.DNA_____________________>

      2960      2970      2980      2990      3000
TAACTCTCCAGTGACAATACCAAGAAGCACACCTTCGCATCGCTCAGTCA
ATTGAGAGGTCACTGTTATGGTTCTTCGTGTGGAAGCGTAGCGAGTCAGT
_____________1 TO 2658 OF GAL4.DNA_____________>

                          >BstAPI
                            |
      3010      3020     |3030      3040      3050
CGCCTTTTCTAGGGCAACAGCAACAGCTGCAATCATTAGTGCCACTGACC
GCGGAAAAGATCCCGTTGTCGTTGTCGACGTTAGTAATCACGGTGACTGG
_____________1 TO 2658 OF GAL4.DNA_____________>

                                                   >SspI
                                                |  3060
                  3070      3080      3090     | 3100
CCGTCTGCTTTGTTTGGTGGCGCCAATTTTAATCAAAGTGGGAATATTGC
GGCAGACGAAACAAACCACCGCGGTTAAAATTAGTTTCACCCTTATAACG
_____________1 TO 2658 OF GAL4.DNA_____________>
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>RsrII
3110 3120 3130 3140| 3150
TGATAGCTCATTGTCCTTCACTTTCACTAACAGTAGCAACGGTCCGAACC
ACTATCGAGTAACAGGAAGTGAAAGTGATTGTCATCGTTGCCAGGCTTGG
1 TO 2658 OF GAL4.DNA
>AfeI >MfeI
3160 3170 3180 3190 3200
TCATAACAACTCAAACAAATTCTCAAGCGCTTTCACAACCAATTGCCTCC
AGTATTGTTGAGTTTGTTTAAGAGTTCGCGAAAGTGTTGGTTAACGGAGG
1 TO 2658 OF GAL4.DNA
>AclI
| 3210 3220 3230 3240 3250
TCTAACGTTCATGATAACTTCATGAATAATGAAATCACGGCTAGTAAAAT
AGATTGCAAGTACTATTGAAGTACTTATTACTTTAGTGCCGATCATTTTA
1 TO 2658 OF GAL4.DNA
>SexAI
3260 3270 3280| 3290 3300
TGATGATGGTAATAATTCAAAACCACTGTCACCTGGTTGGACGGACCAAA
ACTACTACCATTATTAAGTTTTGGTGACAGTGGACCAACCTGCCTGGTTT
1 TO 2658 OF GAL4.DNA
>MluI
3310 3320 3330 3340 3350
CTGCGTATAACGCGTTTGGAATCACTACAGGGATGTTTAATACCACTACA
GACGCATATTGCGCAAACCTTAGTGATGTCCCTACAAATTATGGTGATGT
1 TO 2658 OF GAL4.DNA
3360 3370 3380 3390 3400
ATGGATGATGTATATAACTATCTATTCGATGATGAAGATACCCCACCAAA
TACCTACTACATATATTGATAGATAAGCTACTACTTCTATGGGGTGGTTT
1 TO 2658 OF GAL4.DNA
>AvaI
>BsoBI
>PaeR7I
>PspXI >XbaI
>NotI >TliI >ApaI
>EagI >XhoI >PspOMI| >PmeI
3420 | 3430 |3440 | 3450

```
                CCCAAAAAAAAGAGTAAGCGGCCGCTCGAGTCTAGAGGGCCCGTTTAAACC
                GGGTTTTTTTCTCATTCGCCGGCGAGCTCAGATCTCCCGGGCAAATTTGG
          ___1 TO 2658____>

                     3460       3470       3480       3490       3500
                CGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTG
                GCGACTAGTCGGAGCTGACACGGAAGATCAACGGTCGGTAGACAACAAAC

                     3510       3520       3530       3540       3550
                CCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCC
                GGGGAGGGGGCACGGAAGGAACTGGGACCTTCCACGGTGAGGGTGACAGG

                     3560       3570       3580       3590       3600
                TTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAT
                AAAGGATTATTTTACTCCTTTAACGTAGCGTAACAGACTCATCCACAGTA

                     3610       3620       3630       3640       3650
                TCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA
                AGATAAGACCCCCCACCCCACCCCGTCCTGTCGTTCCCCCTCCTAACCCT

                     3660       3670       3680       3690       3700
                AGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTACTG
                TCTGTTATCGTCCGTACGACCCCTACGCCACCCGAGATACCGAAGATGAC

                     3710       3720       3730       3740       3750
                GGCGGTTTTATGGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCT
                CCGCCAAAATACCTGTCGTTCGCTTGGCCTTAACGGTCGACCCCGCGGGA

                     3760       3770       3780       3790       3800
                CTGGTAAGGTTGGGAAGCCCTGCAAAGTAAACTGGATGGCTTTCTCGCCG
                GACCATTCCAACCCTTCGGGACGTTTCATTTGACCTACCGAAAGAGCGGC

                     3810       3820       3830       3840       3850
                CCAAGGATCTGATGGCGCAGGGGATCAAGCTCTGATCAAGAGACAGGATG
                GGTTCCTAGACTACCGCGTCCCCTAGTTCGAGACTAGTTCTCTGTCCTAC

                                                              >EagI
                                                          | 3860
                          3870       3880       3890       3900
                AGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGG
                TCCTAGCAAAGCGTACTAACTTGTTCTACCTAACGTGCGTCCAAGAGGCC

                     3910       3920       3930       3940       3950
                CCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATC
                GGCGAACCCACCTCTCCGATAAGCCGATACTGACCCGTGTTGTCTGTTAG

                     3960       3970       3980       3990       4000
                GGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGT
                CCGACGAGACTACGGCGGCACAAGGCCGACAGTCGCGTCCCCGCGGGCCA

                     4010       4020       4030       4040       4050
                TCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACG
                AGAAAAACAGTTCTGGCTGGACAGGCCACGGGACTTACTTGACGTTCTGC
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                                                    >FspI
                                                      |
      4060      4070      4080      4090      |  4100
AGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCT
TCCGTCGCGCCGATAGCACCGACCGGTGCTGCCCGCAAGGAACGCGTCGA

      4110      4120      4130      4140      4150
GTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGA
CACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCT

      4160      4170      4180      4190      4200
AGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAG
TCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAACGAGGACGGCTCTTTC

      4210      4220      4230      4240      4250
TATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCT
ATAGGTAGTACCGACTACGTTACGCCGCCGACGTATGCGAACTAGGCCGA

      4260      4270      4280      4290      4300
ACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAC
TGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCGTAGCTCGCTCGTGCATG

    >BsrFI
      |
      4310|     4320      4330      4340      4350
TCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATC
AGCCTACCTTCGGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTCGTAG

      4360      4370      4380      4390      4400
AGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCC
TCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCGAGTTCCGCTCGTACGGG

                  >BtgI
                    |
                  >NcoI
                    |
      4410      4420   |  4430      4440      4450
GACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATAT
CTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATA

                                                >NaeI
                                                  |
                                              >NgoMIV
                                                 | |
                                               >BsrFI
                                                 | |
      4460      4470      4480      4490      | |  4500
CATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGG
GTACCACCTTTTACCGGCGAAAAGACCTAAGTAGCTGACACCGGCCGACC

 >RsrII
   |
      4510      4520      4530      4540      4550
GTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCT
```

```
                CACACCGCCTGGCGATAGTCCTGTATCGCAACCGATGGGCACTATAACGA

                                        >BssSI
                                          |
      4560      4570      4580    |  4590      4600
GAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTAT
CTTCTCGAACCGCCGCTTACCCGACTGGCGAAGGAGCACGAAATGCCATA

      4610      4620      4630      4640      4650
CGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGT
GCGGCGAGGGCTAAGCGTCGCGTAGCGGAAGATAGCGGAAGAACTGCTCA

      4660      4670      4680      4690      4700
TCTTCTGAATTATTAACGCTTACAATTTCCTGATGCGGTATTTTCTCCTT
AGAAGACTTAATAATTGCGAATGTTAAAGGACTACGCCATAAAAGAGGAA

      4710      4720      4730      4740      4750
ACGCATCTGTGCGGTATTTCACACCGCATACAGGTGGCACTTTTCGGGGA
TGCGTAGACACGCCATAAAGTGTGGCGTATGTCCACCGTGAAAAGCCCCT

      4760      4770      4780      4790      4800
AATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATAT
TTACACGCGCCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATA

                                                  >PmlI
                                                | 4810
         4820      4830      4840      4850
GTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATAGCACG
CATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATCGTGC

      4860      4870      4880      4890      4900
TGCTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTT
ACGATTTTGAAGTAAAAATTAAATTTTCCTAGATCCACTTCTAGGAAAAA

      4910      4920      4930      4940      4950
GATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGC
CTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAAGCAAGGTGACTCG

      4960      4970      4980      4990      5000
GTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTC
CAGTCTGGGGCATCTTTTCTAGTTTCCTAGAAGAACTCTAGGAAAAAAAG

      5010      5020      5030      5040      5050
TGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTG
ACGCGCATTAGACGACGAACGTTTGTTTTTTTGGTGGCGATGGTCGCCAC

      5060      5070      5080      5090      5100
GTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGG
CAAACAAACGGCCTAGTTCTCGATGGTTGAGAAAAAGGCTTCCATTGACC

      5110      5120      5130      5140      5150
CTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGT
GAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGATCACATCGGCATCA
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          5160       5170       5180       5190       5200
TAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTG
ATCCGGTGGTGAAGTTCTTGAGACATCGTGGCGGATGTATGGAGCGAGAC
          5210       5220       5230       5240       5250
CTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTAC
GATTAGGACAATGGTCACCGACGACGGTCACCGCTATTCAGCACAGAATG

          5260       5270       5280       5290       5300
CGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCT
GCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGTCGCCAGCCCGA

                        >ApaLI
                              |
          5310    |  5320       5330       5340       5350
GAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACC
CTTGCCCCCCAAGCACGTGTGTCGGGTCGAACCTCGCTTGCTGGATGTGG

          5360       5370       5380       5390       5400
GAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGA
CTTGACTCTATGGATGTCGCACTCGATACTCTTTCGCGGTGCGAAGGGCT

          5410       5420       5430       5440       5450
AGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAG
TCCCTCTTTCCGCCTGTCCATAGGCCATTCGCCGTCCCAGCCTTGTCCTC

                         >BssSI
     |
     | 5460       5470       5480       5490       5500
AGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCT
TCGCGTGCTCCCTCGAAGGTCCCCCTTTGCGGACCATAGAAATATCAGGA

          5510       5520       5530       5540       5550
GTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTC
CAGCCCAAAGCGGTGGAGACTGAACTCGCAGCTAAAAACACTACGAGCAG

          5560       5570       5580       5590       5600
AGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGT
TCCCCCCGCCTCGGATACCTTTTTGCGGTCGTTGCGCCGGAAAAATGCCA

                               >PciI
                                 |
          5610       5620       5630
          TCCTGGGCTTTTGCTGGCCTTTTGCTCACATGTTCTT
          AGGACCCGAAAACGACCGGAAAACGAGTGTACAAGAA
```

(SEQ ID NO.:4)

ABeta 42 Peptide

```
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1                                                               10
15
Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
                     20                                  25
30
Gly Leu Met Val Gly Gly Val Val Ile Ala
                35                      40
```

(SEQ ID NO.:3)

Intra-muscular delivery of aBeta 42 Alzheimer Disease DNA vaccine without the need for gene gun and gold particles.

A major obstacle for commercialization of the AD vaccine has been delivery of the AD vaccine vector under sterile conditions and under practical conditions for patient delivery. The Gene Gun appears problematic with the pharmaceutical industry and the FDA regulatory agency. Other modes of deliver have been addressed by above and by others, such as electroporation, which was been found to be inefficient.

Surprisingly, the inventors have shown herein that intramuscular injection of the AD Single DNA Vector and small concentration of the ABeta 42 peptide elicits very high antibody titer against the ABeta 42 Peptide as indicated in Figure 5.

Figure 5 is a graph that shows the results from 4 muscle injections (once a week (20 ug) with trimer DNA +10 ug Aβ peptide (or separate injection) and tested the antibodies at 6 weeks. It was found that DNA+Peptide without adjuvant elicit a better immune response.

The antibody isotype profile was analyzed to determine the balance of the Th1 / Th2 response. Surprisingly, it was found that the approach of the present invention did not require an adjuvant. A simple, rapid method of injecting the composition taught herein is greatly enhances clinical trials and clinical use for patients.

Isotyping of antibody generated using DNA and abeta 45 peptide delivered. By intra-muscular injection: the trimer single DNA vector (pv1-h3) 20ug + 10 ug abeta 42 peptide were injected into mouse muscle once per week for a total of four weeks. Serum was obtained from the mouse and tested for abeta isotype antibodies at 6 weeks using an ELISA method.

As can be seen in Figure 6, trimer DNA + peptide without adjuvant elicited a better immune response compared to peptide alone. Higher isotype antibodies levels were achieved with the DNA + peptide. Both groups induced the Th1 and Th2 reactions but with a predominance of Th2 (IgG1 and IgG2a).

Figure 6 is a graph that shows the results from 4 muscle injections 4 times (once a week) muscle injection (20 ug Trimer DNA +10 ug Aβ peptide), the serum was tested for Abeta isotype antibodies at 6 weeks with ELISA method. It was found that DNA+Peptide without adjuvant elicited a better immune response compare to peptide alone. Higher isotype antibodies level achieved in DNA+Peptide, but both group induced Th1 and Th2 reaction with predominantly Th2 (IgG1 and IgG2a) bias.

Thus, it was found that the trimer DNA vector can be delivered by intra-muscular injections without the need of the gene gun or gold particles. Furthermore, the levels of antibodies (30 ug/ml) are significantly higher than the DNA (3 ug/ml) or the peptide alone (10 ug/ml) by intramuscular injection or injected intravenously. In addition, the antibody generated was primarily a Th2 response (IgG1 and IgG2a) as indicated in Figure 5.

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. In embodiments of any of the compositions and methods provided herein, "comprising" may be replaced with "consisting essentially of" or "consisting of". As used herein, the phrase "consisting essentially of" requires the specified integer(s) or steps as well as those that do not materially affect the character or function of the claimed invention. As used herein, the term "consisting" is used to indicate the presence of the recited integer (e.g., a feature, an element, a characteristic, a property, a method/process step or a limitation) or group of integers (e.g., feature(s), element(s), characteristic(s), propertie(s), method/process steps or limitation(s)) only.

The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AB, BBC,

AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

As used herein, words of approximation such as, without limitation, “about”, "substantial" or "substantially" refers to a condition that when so modified is understood to not necessarily be absolute or perfect but would be considered close enough to those of ordinary skill in the art to warrant designating the condition as being present. The extent to which the description may vary will depend on how great a change can be instituted and still have one of ordinary skilled in the art recognize the modified feature as still having the required characteristics and capabilities of the unmodified feature. In general, but subject to the preceding discussion, a numerical value herein that is modified by a word of approximation such as “about” may vary from the stated value by at least ±1, 2, 3, 4, 5, 6, 7, 10, 12 or 15%.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method for diagnosis, treatment or prevention of Alzheimer's Disease comprising:

obtaining a biological sample from a subject suspected of having Alzheimer's Disease;

determining the level of expression of HSP 27, wherein a statistically significant increase in HSP27 protein expression in the sample as compared to a sample from a non-Alzheimer's patient is indicative that the subject has Alzheimer's Disease; and

modifying a treatment of the subject as a result of the detection of Alzheimer's Disease by providing the subject with standard therapy or a composition comprising a single vector that expresses an Aβ42 trimer peptide, wherein the composition triggers an immune response to the Aβ42 peptide.

2. The method of claim 1, wherein the composition further comprises an Aβ42 peptide and the DNA vector and the Aβ42 peptide are injected intramuscularly, without the need for a gene gun or gold particles, to trigger an immune response to the Aβ42 peptide.

3. The method of claim 1, wherein the subject is a human.

4. The method of claim 1, wherein the treatment comprises vector of SEQ ID NO: 1 and the peptide of SEQ ID NO: 3.

5. The method of claim 1, wherein the level of HSP 27 is determined by measuring protein expression, and the method is selected from fluorescence detection, chemiluminescence detection, electrochemiluminescence detection and patterned arrays, antibody binding, fluorescence activated sorting, detectable bead sorting, antibody arrays, microarrays, enzymatic arrays, receptor binding arrays, solid-phase binding arrays, liquid phase binding arrays, fluorescent resonance transfer, or radioactive labeling.

6. The method of claim 1, wherein the level of expression of HSP27 is determined at the nucleic acid level, and the method is selected from fluorescence detection, chemiluminescence detection, electrochemiluminescence detection and patterned arrays, reverse transcriptase-polymerase chain reaction, detectable bead sorting, microarrays, enzymatic arrays, allele specific primer extension, target specific primer extension, solid-phase binding arrays, liquid phase binding arrays, fluorescent resonance transfer, or radioactive labeling.

7. The method of claim 1, wherein the level of expression of HSP27 is higher than 85, 90, 95, 100, 110, 115, 120, 125, 130, 145, 150, 275, 300, or 315 ng/ml HSP27 in a blood sample.

8. The method of claim 1, wherein the level of expression of HSP27 is higher than 105, 130, 145, 150, 275, 300, or 315 ng/ml HSP27 in a blood sample.

9. The method of claim 1, wherein the expressed Aβ42 trimer peptide triggers a non-inflammatory IgG1 response.

10. The method of claim 1, wherein the Aβ42 peptide and the expressed Aβ42 trimer peptide are effective to trigger an immune response to the Aβ42 peptide without an adjuvant.

11. A method to evaluate a candidate drug believed to be useful in treating Alzheimer's Disease, the method comprising:

(a) measuring the level of expression of HSP27 from a sample obtained from an Alzheimer's Disease patient;

(b) administering a candidate drug to a first subset of the patients, and a placebo to a second subset of the patients, wherein the candidate drug comprises a single DNA vector encoding an Aβ42 trimer peptide; and

(c) determining if the level of expression of HSP27 or the symptoms of Alzheimer's Disease decreased in the first set of patient as compared to the second subset of patients, wherein a statistically significant decrease is indicative that the candidate drug is useful for treating Alzheimer's Disease.

12. The method of claim 11, wherein the drug candidate further comprises the addition of an Aβ42 peptide, wherein the DNA vector expressing the Aβ42 trimer peptide and the Aβ42 peptide are injected intramuscularly, without the need for a gene gun or gold particles, to trigger an immune response to the Aβ42 peptide.

13. The method of claim 11, wherein the subject is a human.

14. The method of claim 11, wherein the DNA vector encoding the Aβ42 trimer peptide is SEQ ID NO: 1, and the Aβ42 peptide is SEQ ID NO: 3.

15. The method of claim 11, wherein the level of HSP 27 is determined by measuring protein expression, and the method is selected from fluorescence detection, chemiluminescence detection, electrochemiluminescence detection and patterned arrays, antibody binding, fluorescence activated sorting, detectable bead sorting, antibody arrays, microarrays, enzymatic arrays, receptor binding arrays, solid-phase binding arrays, liquid phase binding arrays, fluorescent resonance transfer, or radioactive labeling.

16. The method of claim 11, wherein the level of expression of HSP27 is determined at the nucleic acid level, and the method is selected from fluorescence detection, chemiluminescence detection, electrochemiluminescence detection and patterned arrays, reverse transcriptase-polymerase chain reaction, detectable bead sorting, microarrays, enzymatic arrays, allele specific primer extension, target specific primer extension, solid-phase binding arrays, liquid phase binding arrays, fluorescent resonance transfer, or radioactive labeling.

17. The method of claim 11, wherein the level of expression of HSP27 is higher than 85, 90, 95, 100, 110, 115, 120, 125, 130, 145, 150, 275, 300, or 315 ng/ml HSP27 in a blood sample.

18. The method of claim 11, wherein the level of expression of HSP27 is higher than 105, 130, 145, 150, 275, 300, or 315 ng/ml HSP27 in a blood sample.

19. A vector comprising:

a single nucleic acid that comprises in the following order, a viral gene leader sequence, a Aβ42 trimer sequence, and an endosomal targeting sequence.

20. The vector of claim 19, wherein the viral gene leader sequence is an adenovirus E3 gene leader sequence.

21. The vector of claim 19, further comprises a CMV promoter upstream from the nucleic acid.

22. The vector of claim 19, wherein the vector comprises SEQ ID NO: 1.

23. The vector of claim 19, wherein the endosomal targeting sequence is DXXLL (SEQ ID NO: 2).

24. The vector of claim 19, wherein the vector is PV1-H3 and is adapted for is used to treat or prevent Alzheimer's Disease.

25. A composition for ameliorating the symptoms of Alzheimer's Disease with a composition comprising an Aβ42 peptide and a DNA vector that expresses an Aβ42 trimer peptide in an amount sufficient to ameliorate the symptoms of Alzheimer's Disease, wherein the composition triggers an immune response to the Aβ42 peptide.

26. The composition of claim 25, wherein the DNA vector and the Aβ42 peptide and the DNA vector are injected intramuscularly without the need for a gene gun or gold particles.

27. The composition of claim 25, wherein the Aβ42 peptide is provided at a subtoxic dose.

28. The composition of claim 25, wherein the composition is provided without an adjuvant.

29. The composition of claim 25, wherein the composition leads to a predominantly Th2 response.

30. The composition of claim 25, wherein the peptide comprises SEQ ID NO: 3.

31. The composition of claim 25, wherein the vector comprises SEQ ID NO: 1.

32. The composition of claim 25, wherein the composition consists essentially of the vector of SEQ ID NO: 1 and the peptide of SEQ ID NO: 3.

33. The composition of claim 25, wherein the DNA vector is a single DNA vector.

34. A composition for ameliorating the symptoms of Alzheimer's Disease comprising both an Aβ42 peptide and a DNA vector that expresses an Aβ42 trimer peptide in an amount sufficient to ameliorate the symptoms of Alzheimer's Disease, wherein the Aβ42 peptide and the DNA vector are injected intramuscularly without the need for a gene gun or gold particles, and wherein the composition triggers an immune response to the Aβ42 peptide.

35. The composition of claim 34, wherein the Aβ42 peptide and the DNA vector that expresses the Aβ42 trimer peptide are both provided without an adjuvant.

36. The composition of claim 34, wherein the DNA vector is a single DNA vector.

37. The composition of claim 34, wherein the composition leads to a predominantly Th2 response.

38. The composition of claim 34, wherein the peptide comprises SEQ ID NO: 3.

39. The composition of claim 34, wherein the vector comprises SEQ ID NO: 1.

40. The composition of claim 34, wherein the composition consists essentially of the vector of SEQ ID NO: 1 and the peptide of SEQ ID NO: 3.

41. A method for the treatment or prevention of Alzheimer's Disease comprising injecting both an Aβ42 peptide and a DNA vector that expresses an Aβ42 trimer peptide, wherein the Aβ42 peptide and the DNA vector are adapted for injection intramuscularly without the need for a gene gun or gold particles, wherein the composition triggers an immune response to the Aβ42 peptide.

42. The method of claim 41, wherein the injection triggers a non-inflammatory IgG1 response.

43. The method of claim 41, wherein the Aβ42 peptide and the DNA vector that expresses the Aβ42 trimer peptide are both provided without an adjuvant.

44. The method of claim 41, wherein the DNA vector is a single DNA vector.

45. The method of claim 41, wherein the composition leads to a predominantly Th2 response.

46. The method of claim 41, wherein the Aβ42 peptide comprises SEQ ID NO: 3.

47. The method of claim 41, wherein the vector comprises SEQ ID NO: 1.

48. The method of claim 41, wherein the composition consists essentially of the vector of SEQ ID NO: 1 and the peptide of SEQ ID NO: 3.