Abstract: The present invention relates to a screening method to determine if a candidate nucleic acid sequence effects the production of Aβ protein, the method comprising transfecting a cell, an example of which has been deposited at the ECACC under accession number 07010301, with a candidate nucleic acid sequence and determining if the level of Aβ protein is effected. The present invention also relates to nucleic acid sequences identified by a method of the invention, as well as uses of the nucleic acid sequences for preventing or treating Alzheimer's Disease. The present invention also relates to the use of a cell, an example of which has been deposited at the ECACC under accession number 07010301, to screen for a candidate agent which effects the production of Aβ protein from said cells.
Cell Line for Alzheimer's Disease Therapy Screening

The present invention relates to a screening method to determine if a candidate nucleic acid sequence effects the production of Aβ protein, the method comprising transfecting a cell, an example of which has been deposited at the ECACC under accession number 07010301, with a candidate nucleic acid sequence and determining if the level of Aβ protein is effected. The present invention also relates to nucleic acid sequences identified by a method of the invention, as well as uses of the nucleic acid sequences for preventing or treating Alzheimer's Disease. The present invention also relates to the use of a cell, an example of which has been deposited at the ECACC under accession number 07010301, to screen for a candidate agent which effects the production of Aβ protein from said cells.

Dementia is a brain disorder that seriously affects a person's ability to carry out daily activities. The most common form of dementia among older people is Alzheimer's disease (Alzheimer's Disease), which initially involves the parts of the brain that control thought, memory, and language. There is currently no cure for Alzheimer's Disease.

Alzheimer's Disease begins slowly. At first, the only symptom may be mild forgetfulness, which can be confused with age-related memory change. In the early stage of Alzheimer's Disease, people may have trouble remembering recent events, activities, or the names of familiar people or things. They may not be able to solve simple mathematical problems.

However, as the disease goes on, symptoms are more easily noticed and become serious enough to cause people with Alzheimer's Disease or their family members to seek medical help. Forgetfulness interferes with daily activities. People in the middle stages of Alzheimer's Disease generally forget how to do simple tasks like brushing their teeth or combing their hair. They can no longer think clearly. They begin to fail to recognize familiar people and places. They have problems speaking, understanding, reading, or writing. As the disease progresses, people with Alzheimer's Disease may
become anxious or aggressive, or wander away from home. Eventually, patients need total care.

It is believed that as many as 4.5 million Americans suffer from Alzheimer's Disease (and clearly more world-wide). The disease usually begins after age 60 with the risk increasing with age. While younger people may get Alzheimer's Disease, it is much less common. About 5 percent of men and women ages 65 to 74 have Alzheimer's Disease, and nearly half of those age 85 and older may have the disease. It is important to note that Alzheimer's Disease is not a normal part of aging.

Alzheimer's Disease is named after Dr. Alois Alzheimer, a German doctor. In 1906, Dr. Alzheimer noticed changes in the brain tissue of a woman who had died of an unusual mental illness. He found abnormal clumps (now called amyloid plaques) and tangled bundles of fibers (now called neurofibrillary tangles). Today, these plaques and tangles in the brain are considered signs of Alzheimer's Disease.

Scientists also have found other brain changes in people with Alzheimer's Disease. Nerve cells die in areas of the brain that are vital to memory and other mental abilities, and connections between nerve cells are disrupted. There also are lower levels of some of the chemicals in the brain that carry messages back and forth between nerve cells. Alzheimer's Disease may impair thinking and memory by disrupting these messages.

The amyloid hypothesis postulates that Alzheimer's Disease is caused by aberrant production or clearance of the amyloid β (Aβ) peptide from the brains of affected individuals.

Aβ is toxic to neurons and forms plaques in the brains of Alzheimer's Disease patients. These plaques constitute one of the hallmark pathologies of the disease.
Aβ is produced by the consecutive proteolytic cleavage of the Amyloid Precursor Protein (APP) by β-secretase (BACE) and γ-secretase proteases. APP is also cleaved by α-secretase but this process generates non-amyloidogenic products (see Figure 1).

Cleavage by γ-secretase generates Aβ peptides of variable lengths. The 42 amino acid form of Aβ (AβI-42) is known to be the most toxic.

It has become apparent that in order to perform genomic screening to identify genes involved in the cause of Alzheimer’s Disease, it is desirable (and beneficial) to use a cell line (preferably immortalised) which expresses significant levels of a protein involved in the cause of the disease.

However, genomic screening in cells over-expressing APP is undesirable as this over-expression may significantly alter rate-limiting steps in the process of Aβ production.

Additionally, RNAi knockdown of a significant number of genes affects levels of over-expressed APP transgene (Majercak J, Stone DJ. et al., Proc Natl Acad Sci U S A. (2006) 103(47): 17967-72) causing a high number of false hits and preventing the possibility of screening for genes which modify endogenous APP levels.

Furthermore, the majority of immortalised cell lines used for in vitro experimentation produce undetectable levels of endogenous Aβ production. Cells which do produce detectable endogenous levels of Aβ have, to date, been difficult to transfect with siRNAs using liposomal transfection reagents in a high-throughput paradigm.

The present invention overcomes the problems associated with known cell lines. The present invention provides a method to determine if a candidate nucleic acid sequence effects the production of Aβ protein, the method comprising transfecting a cell with a candidate nucleic acid sequence and determining if the level of Aβ protein is effected. An example of the cell line of the invention has been deposited at the ECACC (located at the Health Protection Agency, Centre for Emergency Preparedness and
The present invention particularly relates to the identification of agents, preferably nucleic acid, which can effect and reduce the activity of Aβ production. Reduction in Aβ production reduces the occurrence of and the problems associated with Alzheimer's Disease. Accordingly, identifying agents which can reduce the production of Aβ is extremely useful for those patients whom suffer from or whom are predisposed to suffer from Alzheimer's Disease.

Preferably, the screening method determines if a candidate nucleic acid sequence decreases the production of Aβ protein. Preferably the candidate nucleic acid is a known gene or region therefrom. The candidate nucleic acid may be siRNA, which is usually 21, 22 or 23 bases in length and is double stranded.

Suitable screens for the present invention are those commonly known in the art and are described, as exemplification of such screens, in the examples section hereinafter. Levels of Aβ can be determined by any means, including the use of various antibody-based detection strategies. These include Western blotting, Homogeneous Time-Resolved Fluorescence (HTRF) or Enzyme linked immunosorbent assay (ELISA), for example as described in the examples section below.

We describe here a human neuroblastoma cell line, ELLIN, which produces clearly detectable levels of Aβ. Aβ production can be clearly inhibited by transfection with siRNA using commercially available liposomal transfection reagents. This cell line is suitable for genomic screening to identify genes potentially involved in endogenous Aβ production (examples, figure 2). ELLIN cells are human neuroblastoma cells. The cells deposited at the ECACC under depositor reference ELLIN are the cell line BE(2)-C. BE(2)-C (ECACC #95011817) is a clonal sub-line of SK-N-BE(2) (ECCAC #95011815) which was isolated from bone marrow of an individual with disseminated neuroblastoma in 1972. They are reported to be multipotential with regard to neuronal enzyme expression and display a high capacity to convert tyrosine
to dopamine. The cells show a small, refractile morphology with short, neurite-like cell processes and tend to grow in aggregates.

A second aspect of the invention relates to an agent which reduces the production of Aβ which is identified by the method of the first aspect of the invention.

Such an agent includes any nucleic acid which is a short interfering nucleic acid (RNA or DNA or other nucleic acid) which is preferably 21, 22 or 23 bases in length, double stranded and has a strand complementary to nucleic acid which encodes a part of the VNIRI, HERC4, GPR12, FLJ38964, FBXO2, FBXL21, KCNJ9, GPR62, GPR34 or GPR101 amino acid sequences referred to herein. Particular short interfering molecules include those described as either siRNA#1, siRNA#2 siRNA#3 or siRNA#4 in the examples section below.

A third aspect of the invention relates to an agent identified according to the second aspects in the invention, for use in treating Alzheimer's Disease.

A fourth aspect of the invention relates to the use of an agent according to the second aspect of the invention, in the manufacture of a medicament for treating Alzheimer's Disease.

A fifth aspect of the invention relates to a method of treating a patient who suffers from or whom is predisposed to suffer from Alzheimer's Disease, the method comprising screening a candidate agent according to the first aspect of the invention and administering the agent to the patient. Preferably, the patient is in need of treatment for Alzheimer's Disease. A sixth aspect of the invention relates to a method of treating a patient who suffers from or whom is predisposed to suffer from Alzheimer's Disease by administration of a therapeutic agent, wherein the therapeutic agent is obtained from a method according to the first aspect of the invention. Again, preferably the patient is in need of treatment. Treatment can be therapeutic or prophylactic.
All preferred embodiments of the first to fifth aspects of the invention, also apply to each other aspect, *mutatis mutandis*.

The present invention is described with reference to the following figures, in which:

**Figure 1** illustrates processing of the Amyloid Precursor Protein (APP) by β, α and γ-secretase. Cleavage of APP by β-secretase (BACE) generates soluble APPβ (sAPPβ) and a membrane-bound C-terminal fragment, βCTF. The β CTF is cleaved by γ-secretase to generate Aβ and the APP intracellular domain (AICD) fragment.

Cleavage of APP by β-secretase generates soluble APPβ (sAPPβ) and a membrane-bound C-terminal fragment, βCTF. The βCTF is cleaved by β-secretase to generate the non-amyloidogenic peptide P3 and the APP intracellular domain (AICD) fragment;

**Figure 2a** illustrates inhibition of endogenous Aβl-40 production in ELLIN human neuroblastoma cells by siRNA-mediated gene knockdown. siRNAs targeting VNIR1, HERC4, GPR12, FLJ38964, FBXO2, FBXL21, KCNJ9, GPR62, GPR34 and GPR101 decreased production of Aβl-40 in ELLIN human neuroblastoma cells;

**Figure 2b** illustrates inhibition of endogenous APP and APP C-terminal fragment levels in ELLIN human neuroblastoma cells by GPR12 and FLJ38964 knockdown. ELLIN cells were transfected with anti-FLJ38964 and anti-GPR12 siRNAs. Both full-length APP and APP C-terminal fragment levels were analysed by Western blotting using an anti-APP antibody (1:2000 Invitrogen #51-2700) demonstrating siRNA-mediated inhibition of APP levels. N-Cadherin levels were unaffected as demonstrated by probing with an anti-N-Cadherin antibody (1:2000 BD Biosciences #610920);

**Figure 3** illustrates VNIR1 amino acid sequence. VNIR1 sequence 1: Protein_ID: NP_065684.1 from nucleotide REFSEQ accession NM_020633.2;
Figure 4 illustrates HERC4 isoform amino acid sequences. HERC4 sequence 1: Protein_ID: NP_071362.1 from nucleotide REFSEQ accession NM_022079.1 - Isoform A;

Figure 5 illustrates HERC4 sequence 2: ProteinID: NP_056416.2 from nucleotide REFSEQ accession NM_015601 - Isoform B;

Figure 6 illustrates GPR12 amino acid sequence. GPR12 sequence 1: Protein ID: NP_005279.1 from nucleotide REFSEQ accession NM_005288.1;

Figure 7 illustrates FLJ38964 amino acid sequence. FLJ38964 sequence 1: Protein ID: NP_775798.1 from nucleotide REFSEQ accession NM_173527.1. [ ] = potential additional sequence. FLJ38964 is a hypothetical protein and the start codon of the gene has not been experimentally determined;

Figure 8 illustrates FBXO2 amino acid sequence. FBXO2 sequence 1: Protein ID: NP_036300.2 from nucleotide REFSEQ accession NM_012168.4;

Figure 9 illustrates FBXL21 amino acid sequence. FBXL21 sequence 1: ProteinID: NP_036291.1 from nucleotide REFSEQ accession NM_012159.1;

Figure 10 illustrates KCNJ9 amino acid sequence. KCNJ9 sequence 1: Protein ID: NP_004974.2 from nucleotide REFSEQ accession NM_004983.2;

Figure 11 illustrates GPR62 amino acid sequence. GPR62 sequence 1: Protein ID: NP_543141.2 from nucleotide REFSEQ accession NM_080865.2;

Figure 12 illustrates GPR34 amino acid sequences. GPR34 sequence 1: Protein ID: NP_005291.1 from nucleotide REFSEQ accession NM_005300.2 - Isoform 1;

Figure 13 illustrates GPR34 sequence 2: Protein ID: NP_001028685.1 from nucleotide REFSEQ: accession NM_001033513.1 - Isoform 2;
Figure 14 illustrates GPR34 sequence: Protein H): NP_001028686.1 from nucleotide REFSEQ: accession NM_001033514.1 - Isoform 3 and

Figure 15 illustrates GPR1Ol amino acid sequence. GPR1Ol sequence: Protein ID: NP_473362.1 from nucleotide REFSEQ accession NM_054021.1.

**Experimental details**

**ELLIN growth conditions**

ELLIN cells were cultured in a humidified atmosphere of 10% CO2 at 37°C in a 1:1 mix of Minimum Essential Medium (Sigma-Aldrich #M2279) and HAM'S F12 medium (Invitrogen #21765-029) supplemented with 15% (v/v) bovine foetal calf serum (PAA laboratories #A15-003), a 1:100 dilution of IOOX Non-Essential Amino Acids (Sigma-Aldrich #M7145), 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen #15140-1 14).

**Examples**

RNA interference (RNAi) is a recently discovered functional tool. This is a phenomenon where an RNA introduced into a cell ultimately causes the degradation of the complementary cellular mRNA, and leads to the knockdown of gene activity (*Hammond S, Harmon G, et al., Nature (2000) 404(6775):293-6*).


**siRNA screening protocol**
ELLIN cell stocks were trypsinised (Invitrogen #25300-054) and replated 1:2 the day before transfection. On the day of transfection cells were again trypsinised and subsequently replated in serum and antibiotics free media at $3.75 \times 10^4$ cells per 80μl media per well of a 96-well plate.

Transfection reaction complexes were prepared in OptiMEM (Invitrogen #51985-026) using either 1μl of Oligofectamine (Invitrogen #11668-027) or 0.2μl Dharmafect 4 (Dharmacon # T-2004-02) per well of cells to be transfected. Under standard conditions siRNAs were screened at 100nM final concentration on the cells.

A randomised non-targeting negative control siRNA oligonucleotide was purchased from Dharmacon (#D-001810-01-05).

Plated cells were incubated with transfection reactions for 4 hours. The serum free media on the cells was then made up to a final concentration of 5% (v/v) bovine foetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin.

72 hours after transfection cell media were harvested for analysis of Aβ levels. Aβ1-40 levels were determined using an ELISA kit purchased from Biosource (#KHB3482). Cell viability was determined using Alamar Blue (Biosource #DAL1025).

Cell lysates were also harvested and both full-length APP and APP C-terminal fragment levels analysed by Western blotting using an anti-APP antibody (1:2000 Invitrogen #51-2700).

**Example 1: Assaying of anti-VNIRI siRNAs in ELLIN cells**

Annealed anti-VNIRI siRNA oligonucleotides were purchased from Dharmacon (#MU-013 177-00).
Sense oligo: 5’-GGGAUUAGCUUCCUCAUUCUUS’
Antisense oligo: 5’-P-GAAUGAGGAAGCUAAUCCUU-S’

siRNA#2
Sense oligo: 5’- CAACUGGCCUUGGCUAACUUU-3’
Antisense oligo: 5’-P- AGUUAGCCAAGGCCAGUUGUU-3’

**Example 2: Assaying of anti-HERC4 siRNAs in ELLIN cells**

Annealed anti-HERC4 siRNA oligonucleotides were purchased from Dharmaco (#MU-021426-00).

siRNA#1
Sense oligo: 5’-GCAGAAACAUCCUACGAAAUU-S’
Antisense oligo: 5’-P-UUAUCGUAGGAGUUGUCUUCUU-3’

siRNA#2
Sense oligo: 5’- GUACAGCUCUUGUGAACCUUU-3’
Antisense oligo: 5’-P-AGGUUCACAAGAGCGUGUACUU-S’

siRNA#3
Sense oligo: 5’- GAAAUUGGUACCCCUAUAAUU-3’
Antisense oligo: 5’-P-UUAUAGGGGUACCAAUUCUUS’

**Example 3: Assaying of anti-GPR12 siRNAs in ELLIN cells**

Annealed anti-GPR12 siRNA oligonucleotides were purchased from Dharmaco (#MU-005538-00).

siRNA#1
Sense oligo: 5’-UCAGACCUCUCACCAAGAAUU -3’
Antisense oligo: 5’-P-UUCUUGGUGAGCGGUCGAAU -3’
siRNA#2
Sense oligo: 5'-GAGAGGACGGUCACGUUUAUU-S'
Antisense oligo: 5'-P-UAAACGUGACCGUCCUCUU-3'

Example 4: Assaying of anti-FL.T38964 siRNAs in ELLIN cells

Annealed anti-FLJ38964 siRNA oligonucleotides were purchased from Dharmacon (#MU-008318-00).

siRNA#1
Sense oligo: 5'-GCAGAUGCCACGUACCUAUS'-
Antisense oligo: 5'-P-UUAGUAGCGUGCAUCUCCUU-S'

siRNA#2
Sense oligo: 5'-GGCACUGCCUGGAGACGAGUUU-3'
Antisense oligo: 5'-P-ACUGUCUCAGGCAUCGCUU-3'

Example 5: Assaying of anti-FBXO2 siRNAs in ELLIN cells

Annealed anti-FBXO2 siRNA oligonucleotides were purchased from Dharmacon (#MU-012429-00).

siRNA#1
Sense oligo: 5'- GGACUGCCUGGAGACGAGUUU-3'
Antisense oligo: 5'-P-ACUGUCUCAGGCAUCGCUU -3'

siRNA#2
Sense oligo: 5'- GGACUGCCUGGAGACGAGUUU-3'
Antisense oligo: 5'-P-ACUGUCUCAGGCAUCGCUU -3'

siRNA#3
Sense oligo: 5'- AUGAGAGCGUCAAGAAGUAi π J-3'  
Antisense oligo: 5'-P-UACUUCUUGACGCUCUCAUUU-S'

siRNA#4

5 Sense oligo: 5'- CCGUUAAGCUACUGUCCGAU-3'  
Antisense oligo: 5'-P-UCCGACAGUACUAAACGGUU-S'

Example 6: Assaying of anti-FBXL21 siRNAs in ELLIN cells

Annealed anti-FBXL21 siRNA oligonucleotides were purchased from Dharmacon (#MU-012423-00).

siRNA#1

Sense oligo: 5'-GGGAUGCACUUAUUAAACAUU -3'  
Antisense oligo: 5'-P- UGUUUAUAAGUGCAUCCCUU-3'

siRNA#2

Sense oligo: 5'- CAACUGUCCUCGACUGAUUUU-3'  
Antisense oligo: 5'-P-AAUCAGUCAGGACAGUUGUU-S'

Example 7: Assaying of anti-KCNJ9 siRNAs in ELLIN cells

Annealed anti-KCNJ9 siRNA oligonucleotides were purchased from Dharmacon (#MU-006253-00).

siRNA#1

Sense oligo: 5'-GCAACGUGCAGCAGGGCAAUU-S'  
Antisense oligo: 5'-P-UUGCCCUGCUGCACGUUGCUU-S'

siRNA#2

Sense oligo: 5'-GCAACGUGCAGCAGGGCAAUU-S'  
Antisense oligo: 5'-P-UUGCCCUGCUGCAGUUGCUU-S'
siRNA#3
Sense oligo: 5'-UCGUUAUCCUCGAGGGCAUUU-S'
Antisense oligo: 5'-P-AUGCCCUCGAGGAUAACGAUU-S'

siRNA#4
Sense oligo: 5'-GCGUGGACGCGUCGCGUCAUU-S'
Antisense oligo: 5'-P-UGACGCACGCGGCUCCACGCUU-S'

Example 8: Assaying of anti-GPR62 siRNAs in ELLIN cells

Annealed anti-GPR62 siRNA oligonucleotides were purchased from Dharmacon (#MU-005586-00).

siRNA#1
Sense oligo: 5'-GGACAAAGCUACUGAAACUUU-S'
Antisense oligo: 5'-P-AGUUUCAGUAGCUUUGUCCUU-S'

siRNA#2
Sense oligo: 5'-CCUAAGGGCUCACAAACAAUU-3'
Antisense oligo: 5'-P- UUGGUUGUGAGCCCUUAGGUU-S'

siRNA#3
Sense oligo: 5'-GCCACAACACCAGUAUUUU-S'
Antisense oligo: 5'-P- AAAUACUGGUGUUGUGGCUU-3'

Example 9: Assaying of anti-GPR34 siRNAs in ELLIN cells

Annealed anti-GPR34 siRNA oligonucleotides were purchased from Dharmacon (#MU-005566-00).

siRNA#1
Sense oligo: 5'-GGAAGAAUCUAUUGAGGAUUU-S'  
Antisense oligo: 5'-P-AUCCUAAUAGAUUCUUCCU-S'

**siRNA#2**  
5' Sense oligo: 5'-GAACAUAAUCGCCCUCUAUUU-3'  
Antisense oligo: 5'-P-AUGAGGGCGAUUAUGUUCU-S'

**Example 10: Assaying of anti-GPR101 siRNAs in ELLIN cells**

10 Annealed anti-GPR101 siRNA oligonucleotides were purchased from Dharmacon (#MU-005526-01).

**siRNA#1**  
Sense oligo: 5'-GCACCAAAGUUGAGGAGAAUU-S'  
Antisense oligo: 5'-P-UUCUCCUACUUUGUGCUUU-3'

**siRNA#2**  
Sense oligo: 5'-GAACAGAGGGUGGGACUGAUU-S'  
Antisense oligo: 5'-P UCAGUCCCACCCUCUGUUCU-S'

**siRNA#3**  
Sense oligo: 5'-GCAAGAUUGUCCCUUCCUAUU-S'  
Antisense oligo: 5'-P-UAGGAAGGGACAAUCUUGCUU-S'

**siRNA#4**  
Sense oligo: 5'- GUCAGUGGAUCGCUCUUGUUU-3'  
Antisense oligo: 5'-P-CAAGUAGCGACUCACUGACU-S'

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. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. 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.
## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule \3bis)

### A. The indications made below relate to the microorganism referred to in the description on page 3 and 4, line 28-2.

<table>
<thead>
<tr>
<th>Name of depositary institution</th>
<th>European Collection of Cell Cultures (ECACC)</th>
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<tbody>
<tr>
<td>Address (including postal code and country)</td>
<td>Health Protection Agency Centre for Emergency Preparedness and Response Porton Down Salisbury, Wiltshire SP4 OSG United Kingdom</td>
</tr>
<tr>
<td>Date of deposit</td>
<td>3 January 2007</td>
</tr>
<tr>
<td>Accession Number</td>
<td>07010301</td>
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</tbody>
</table>

### B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet.

### C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet.

### D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

In respect of all designated states to which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited biological material be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g., EPC Rule 32, UK Patent Rules 2007, Schedule 1, paragraph 6 and generally similar provisions mutatis mutandis for any other designated state.

### E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")
Claims

1. A screening method to determine if a candidate nucleic acid sequence effects the production of Aβ protein, the method comprising transfecting a cell, an example of which has been deposited at the ECACC under accession number 07010301, with a candidate nucleic acid sequence and determining if the level of Aβ protein is effected.

2. A screening method, as claimed in claim 1, to determine if a candidate nucleic acid sequence decreases the production of Aβ protein.

3. A nucleic acid sequence identified by a method as claimed in claim 2.

4. A nucleic acid sequence as claimed in claim 3, for use in treating Alzheimer's Disease.

5. Use of a nucleic acid as claimed in claim 3, in the manufacture of a medicament for treating Alzheimer's Disease.

6. A method of treating a patient who suffers or is predisposed to suffer from Alzheimer's Disease, the method comprising screening a candidate nucleic acid, as claimed in claim 1 or claim 2 to identify an agent which reduces the production of Aβ protein and administering the agent to the patient.

7. A method of treating a patient who suffers from or who is predisposed to suffer from Alzheimer's Disease, by administration of a nucleic acid wherein the nucleic acid is obtained by a method as claimed in claim 1 or claim 2.

8. Use of a cell, an example of which has been deposited at the ECACC under accession number 07010301, to screen for a candidate agent, which effects the production of Aβ protein from said cell.
FIG. 1
<table>
<thead>
<tr>
<th>siRNA</th>
<th>ABETA1-40 % Mock (normalized to Cell Viability)</th>
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<tbody>
<tr>
<td>Mock</td>
<td></td>
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<tr>
<td>Non-targeting control</td>
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<tr>
<td>siRNA #3</td>
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</tr>
</tbody>
</table>

**FIG. 2a**

Effects of Transfected siRNAs on ABeta 1-40 Production in ELL1 T Cells
FIG. 2b

- Amyloid Precursor Protein
- β C-Terminal Fragment
- α C-Terminal Fragment
- N-Cadherin
FIG. 3

FIG. 4

SUBSTITUTE SHEET (RULE 26)
FIG. 5

MLCWGNASFGQLGLGLIGIDEEIVLEPRKSDFFINKVRDVCGLRHIVLVFLDDGTYT
CGCNLGLQGLGHEKSRRKPEQVVALQAQNIAVAVSCGAEHTLALNDKGGQYAWGLSDG
QLGLVSGECIRPVPRNISLSDIQIVQVACGYHSLSALKSVEFVCWGCQNKYGQLGL
GTDCKKQTSPQLLSLLGIPFMQVAAGGASHFVLTSLSGAIFGWGRRNKFGQLGLNDE
DRYVPLNLKLSRSQKIVYVICGEDHTAILTKEGGVFTFGAGYQQLQHNGTSHEINP
RKVFELMGSIIVTEIAACRQHTSAFVPSSGRIYSFGLGLGGQQLGTGSTSNRKPSTFTVK
GNWYPYNGQCLPIPDDSEEFVCKRIFSFGDGQSFHYSSPQNCGGPPDDFRCNPNTQKI
WTVEALIIQKWSYPSGRFPVEIANEIDGTFSSSGCNLNSFLAVSNDDHYRTGFRS
GVDMAAARRLLFHKLQPDHPQISQVQVAASLEKLIPKLTSSLPDVEALRFYTLTPEC
PLMSDNSNFTTIAIPFGTALVNLEAKPLKVLNENWSVLEPLEPLKLIVIELKEVVHLL
LKLKYKIGIPSPERRIFNSFLHTALKVIEILHRVNEKMGQIIQYDKFYIHVEQELID
RNDYINWVQQQAYGMLADIPVICTIPVFVFDQAKTTLLLQTDAVLQMIAQDAHRQQ
NVSSLLFLPVEISVNPLCLILVVRREIVNGDAEVLRKTNIDKKLPIKVFVGEDADV
AGGVRKKEEFFLLIMRELLLDPKGMFRYEDRSRLIFDSKDFEDSDFLHQILIGCILAI
YNTCIVDLHFFLALYKYKLLKKKPSLDLDEMPDVRGSMQQLDLDDYPEDDIEETFCLN
FTITVENFGATEVKELVNGADATTAVKNQNRQEFVDAYVDIFNKSASLFDHAFHGF
HKVCVGKVLLLFQPQNELQAMVYNTNYDKLEELKNTYEKGYEWAEPHTIKIFVEVHF
ELPLEKQKQFLLFTGSDRPIQLGMKSLKLVQSTGGGEEYLPVSHTCFNLLDLPKY
TEKETLRKLIQAID

FIG. 6

MNEDLKVNLSGLPRYLDAAANAENISAASAASRSVVPAVEPEPFLVNPVIDVLCCTSGL
ISCENAIYVIIFHNPSLRAPFLLIGSLALADLLAGILITNFVNYFQLEATKL
VTIGLIVASFASSACVSSLLAITVDRYLSLLYALTYYHSETVTFTTYVMLVMWGTSICL
GLLPVMNGWCLRDESTSCVPRPLKNNAILSVSFLMFALMLQLYIQICLIVMRHA
HQIALQHHFLATSHYVTTRKGVSTLAILGTFACAWMPFTLYSLIADYTPSIYTYA
TLPATYNSINPVYAFRNSQIEQKALCLICCGCIPSSLARSPSDV
FIG. 7

FIG. 8

FIG. 9
MAQENAAFSPGQEEPPRRRRGRQYVEKDGRCNVQQGVNRTYRLDLFTDVTLLDWLQWR\RLSSLFFLAVALTWLFFGAIWWLIAYGRGDLHLEDTAWTCPVNVNLNFVAAFLFS\IETETTGYGHRVITDQCPGIVLQLLQAILGSMNVAFMVCMFVVKISQPNRRA\ALTFVSSHAVVSLRDGRLCMFGRGDLRSSHIVEASIRAKLIRSRQTELGEIFI\PLHTDL\SVGFTDGDDLRLFVSPVLSHEIDAASPFWEAASRAENGDFREIEVILEGMEAGTM\TCQRASSYLVDEVVSHGRTSFLVLTEDFGEVYASFHETFREVPSCSARELAEEA\ARLDAHLYWSIPSLDEKEVVEEGAGEGGAGEAGADKEQNGCLPFPSES

FIG. 10

MANSTGLNASEVAGSLGLLALAVVEVEGALLNGALLLVLRTGLRDALYLAHICVV\DLILAAASIMPLGLLLAAPPPGLGRVRGPAAPCRARFSAALPACTLGVAPAILGLARY\RLIVHPLRPSRRPVPVLTLAVWAAGGLLLGALSLGPAPPPAPAPARCSVLAGGLYG\FRPLWALLAFALPALLLLSAGYGGIFVVARAAALRPPARPSRLRSDSRLSRLSILP\PLRPRPLPGKAAALAPALAVQGFAQACWLPYGCACLAPAARAEEAEEAVTWSVAYSAFAA\HPFLYGLLQRPVRVLALGRSLRALLPGPVRACTPQAWHRALLQLRQPPEPGPAVGPE\EAEPEQTPELAGGRSPAYQGPPESSLS

FIG. 11

MRSHTITTTTTSVSSPYSSHMRFITNHDQPPQNFATPNVTTCPMDEKLLSTVL\TTTSYIVFFIVGLVGNIAVLYVFLGHIHRKNSIQIYLLNFLVAIADLLLIFCLPFPRIMYH\INQNKTGLVILCVKVGTLFYMNNYISIILLGFISLDRYIKINRSIQKRAITTQS\IYVCCIVWMLALGGFLTMIILTLKKGTHNSTMCFHYRDKNAGKEAIENFILVVMFW\LIFLLIIILSYIKGCNLLRISRKRSSFKPNPSKGYATTANSFIVLIITFCVYHAF\RPIYITSSQLNVSSCWWKEIVHTNEIMVLSFNCVDVMYFLMSSNIRKIMCQLL\FRRFQGEPURSESTSFKPGYSLHDTOVAVKIQSSSKST

FIG. 12