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(71) Applicant(s):
University Of Sheffield
(Incorporated in the United Kingdom)
Western Bank, Firth Court, Sheffield, S10 2TN,
United Kingdom

(72) Inventor(s):
Guillaume Hautbergue
Mimoun Azzouz
Pamela Shaw

(74) Agent and/or Address for Service:
Symbiosis IP Limited
Innovation Centre, Innovation Way, Heslington, York,
YO10 5DG, United Kingdom

(51) INT CL:
C12N 15/864 (2006.01) ***A61K 31/713*** (2006.01)
A61K 38/16 (2006.01) ***A61K 38/17*** (2006.01)
A61P 25/28 (2006.01) ***C07K 14/47*** (2006.01)
C12N 15/113 (2010.01)

(56) Documents Cited:
WO 2017/161273 A1 **WO 2014/007858 A1**
US 20190194660 A1

(58) Field of Search:
Other: **SEARCH-PATENT, SEARCH-NPL, Cas Online**

(54) Title of the Invention: **Viral vector**
Abstract Title: **Antagonists of Serine/Arginine Rich Splicing Factor 1 (SRSF1)**

(57) The present disclosure relates to antagonists that target or inhibit Serine/Arginine Rich Splicing Factor 1 (SRSF1). A viral vector, such as an AAV, is disclosed which comprises a non-expressed sequence (e.g. a stuffer sequence) and a nucleic acid encoding an antagonist of SRSF1 which is operably linked to a promotor. The antagonist may be an inhibitory RNA, such as shRNA, miRNA, antisense RNA or may be an inhibitory peptide which is, for example, fused to a cell penetrating peptide, CPP. The viral vector may be formulated as a pharmaceutical. Also disclosed are an inhibitory RNA to SRSF1, a cell penetrating peptide antagonist of SRSF1 and an agent comprising an inhibitory RNA to SRSF1, each of which may be formulated into a pharmaceutical composition. The SRSF1 antagonists may be used in the treatment of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS, motor neurone disease, MND), sporadic Amyotrophic Lateral Sclerosis which is not caused by a pathological C9ORF72 hexanucleotide repeat expansion, sporadic frontotemporal dementia (FTD) or Fragile X-associated tremor/ataxia syndrome (FXTAS).

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Figure 1

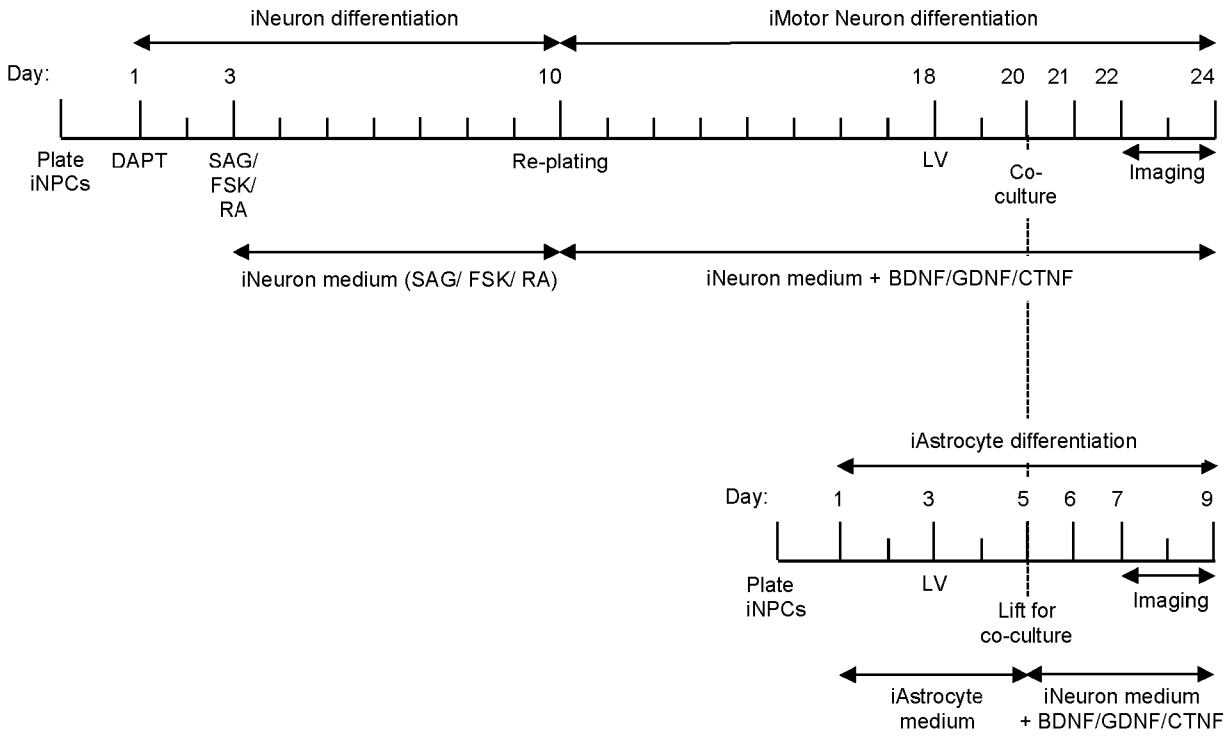


Figure 2

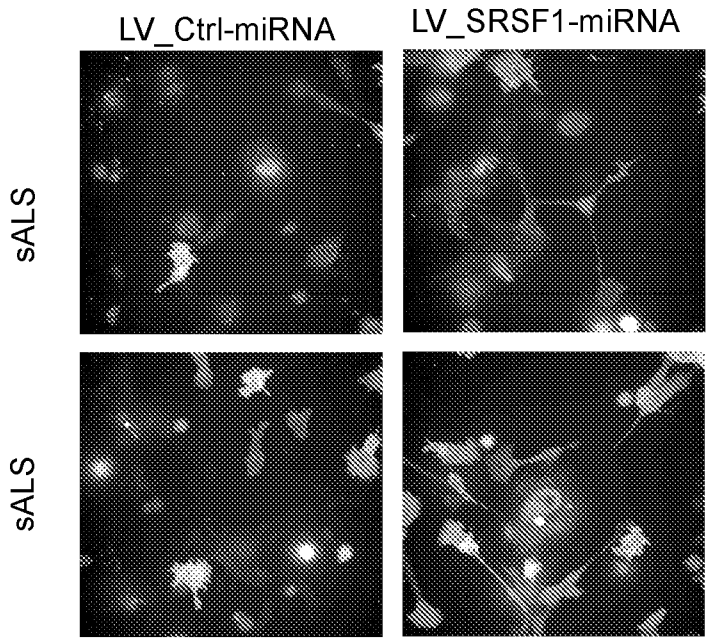


Figure 3

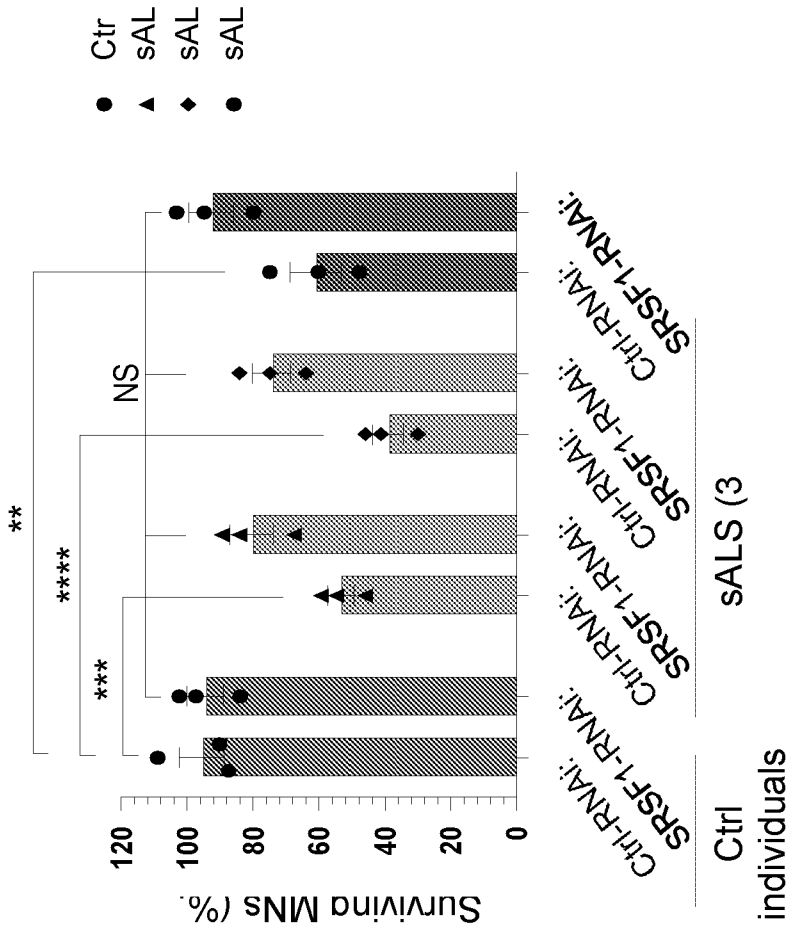


Figure 4

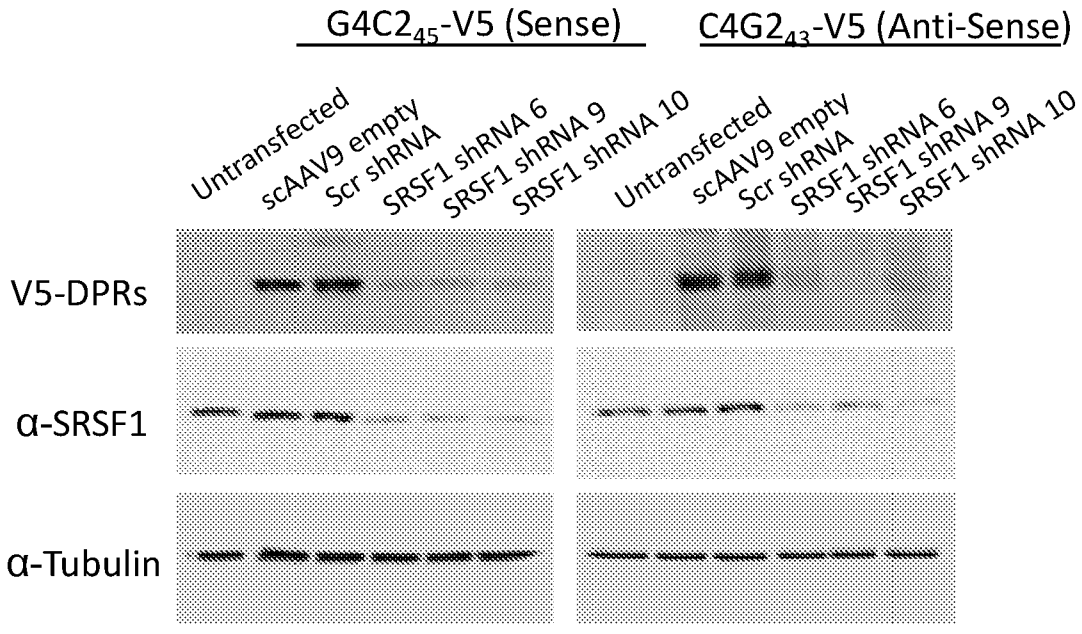


Figure 5

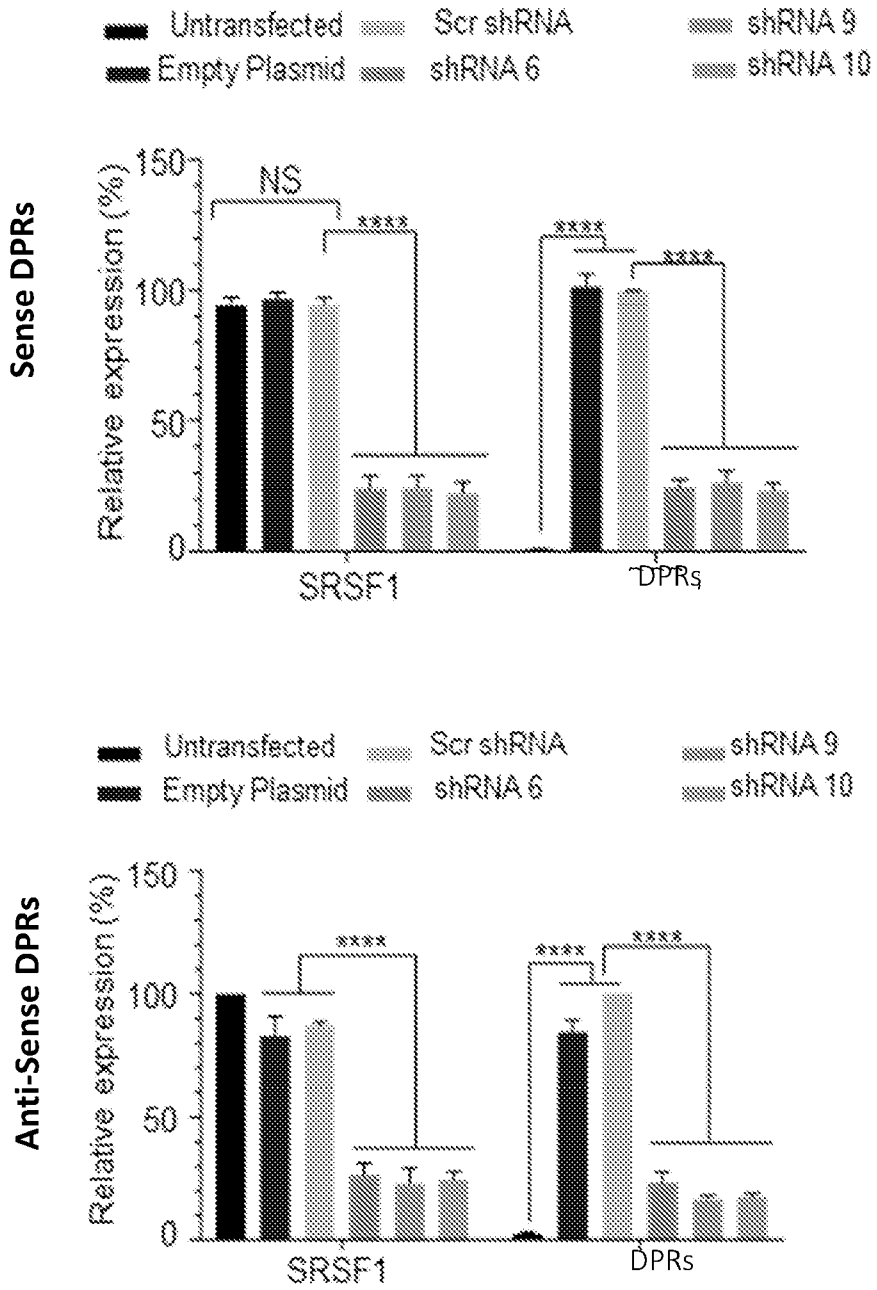

Untransfected
Empty Plasmid
Scr shRNA
shRNA 6
shRNA 9
shRNA 10
Sense DPRs
Relative expression (%)
150
100
50
0
NS
****
****
****
SRSF1
DPRs
Untransfected
Empty Plasmid
Scr shRNA
shRNA 6
shRNA 9
shRNA 10
Anti-Sense DPRs
Relative expression (%)
150
100
50
0
****
****
****
SRSF1
DPRs

Figure 6

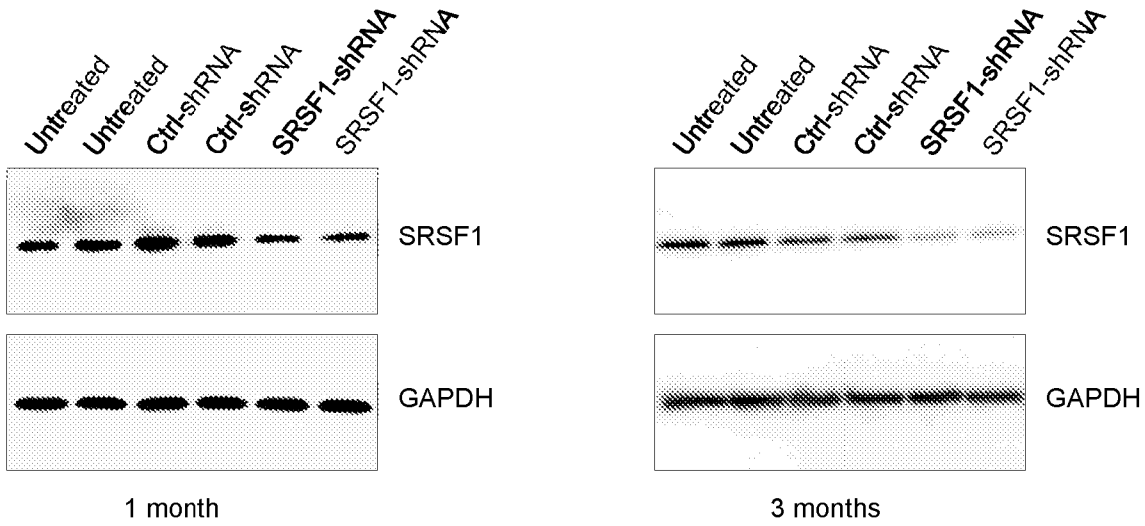
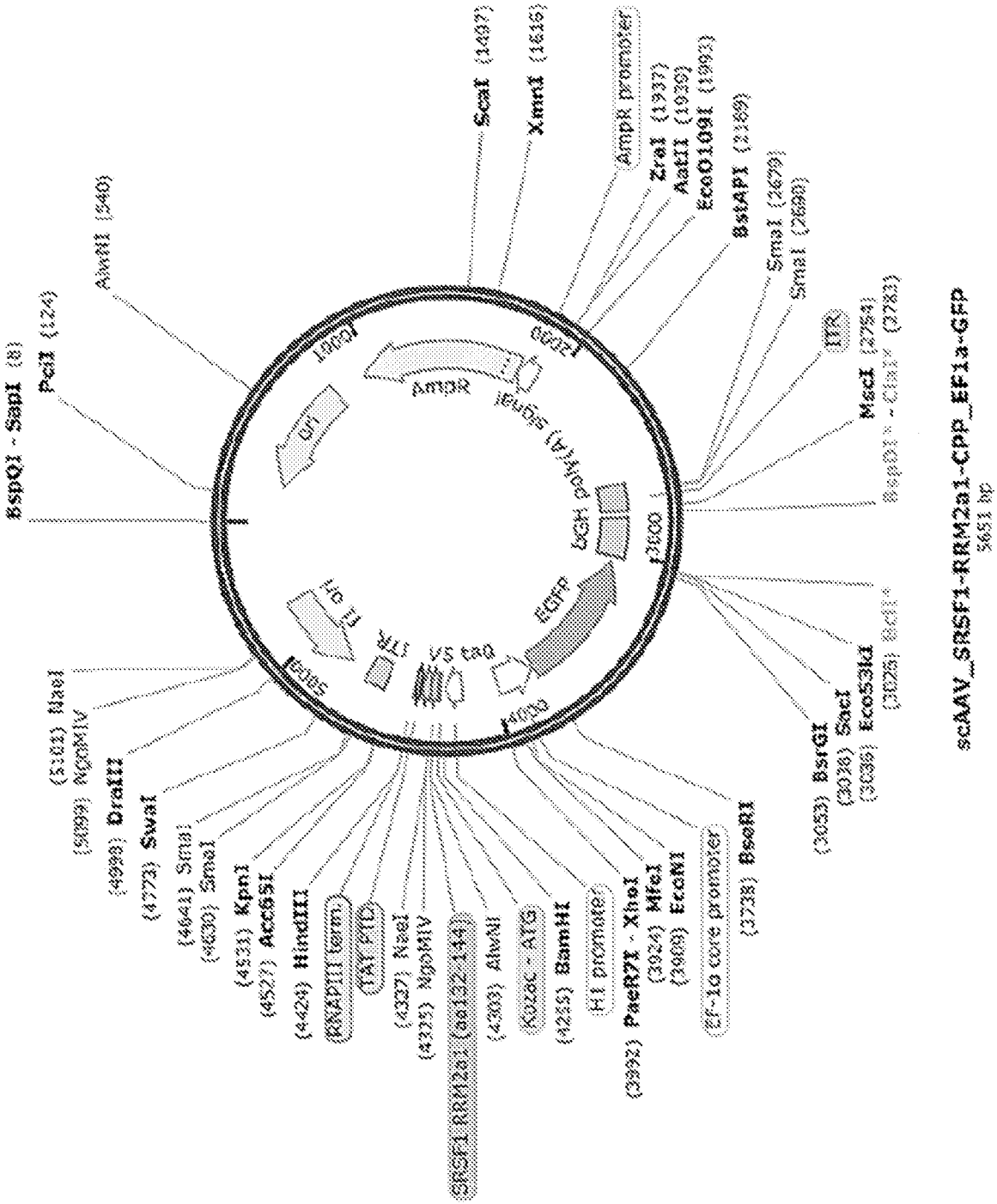

Untreated
Untreated
Ctrl-shRNA
Ctrl-shRNA
SRSF1-shRNA
SRSF1-shRNA
SRSF1
GAPDH
1 month
Untreated
Untreated
Ctrl-shRNA
Ctrl-shRNA
SRSF1-shRNA
SRSF1-shRNA
SRSF1
GAPDH
3 months

Figure 7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5651 bp
BspQI - SapI (8)
PciI (124)
AlwNI (540)
ScaI (1497)
XmnI (1616)
AmpR promoter
ZraI (1937)
AatII (1939)
Eco0109I (1962)
BstAPI (2269)
SmaI (2679)
SmaI (2680)
ITR
MscI (2784)
BspQI - ClaI (2783)
BsrGI (3053)
SacI (3076)
Eco53kI (3086)
BseRI (3738)
EF-1α core promoter
PaeR7I - XhoI (3902)
MfeI (3924)
EcoNI (3980)
H1 promoter
BamHI (4235)
Kozac - ATG
AlwNI (4303)
SRSF1 RRM2a1 (aa132-144)
NgoMIV (4315)
NaeI (4327)
TAT PTD
RNAPIII term.
HindIII (4424)
AccGI (4521)
KpnI (4531)
SmaI (4630)
SmaI (4641)
SwaI (4772)
DraIII (4928)
NgoMIV (5099)
HaeI (5101)
f1 ori
ITR
V5 tag
EGFP
bGH poly(A) signal
f2ori
AmpR
pUC ori

Figure 7 continued

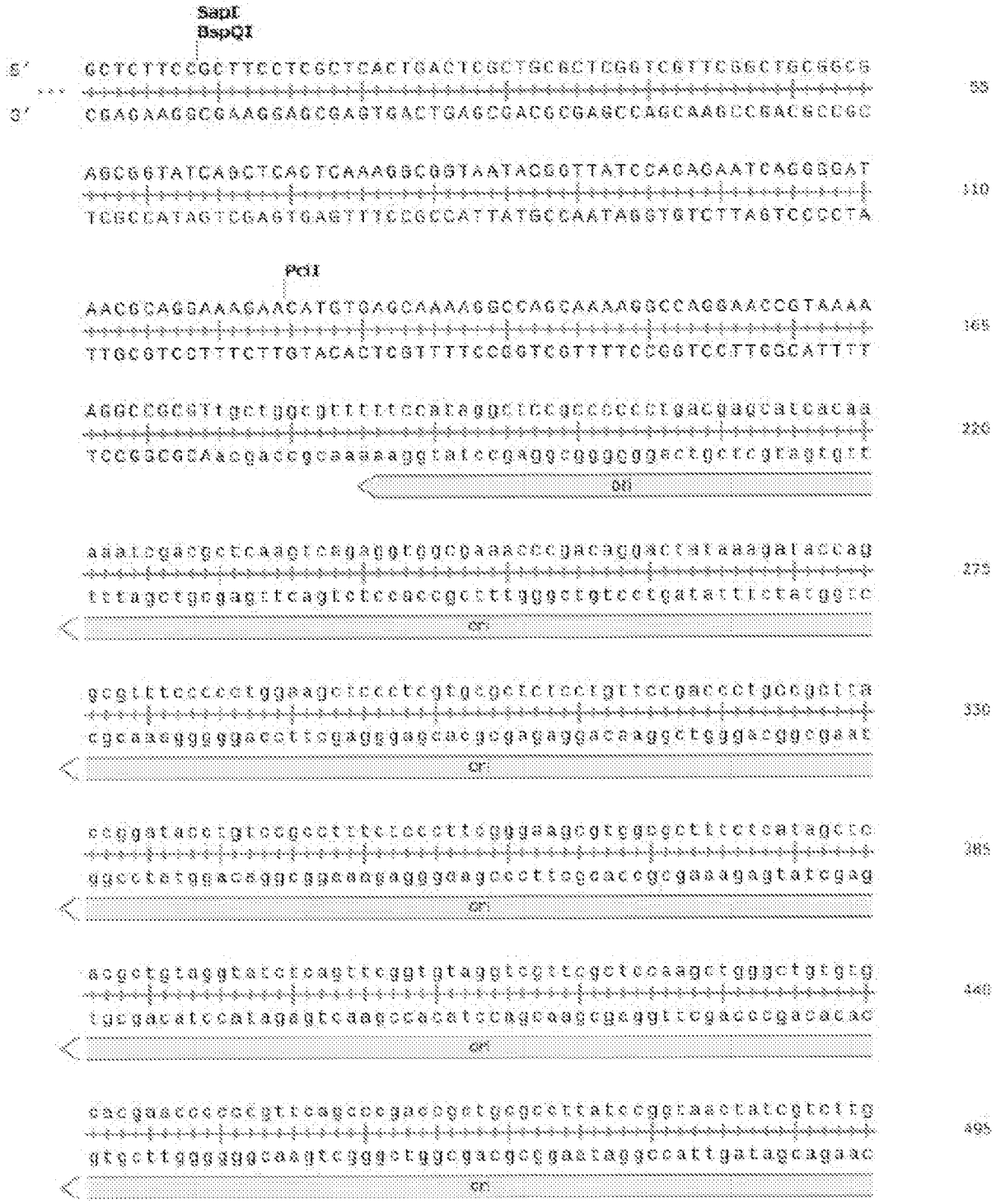
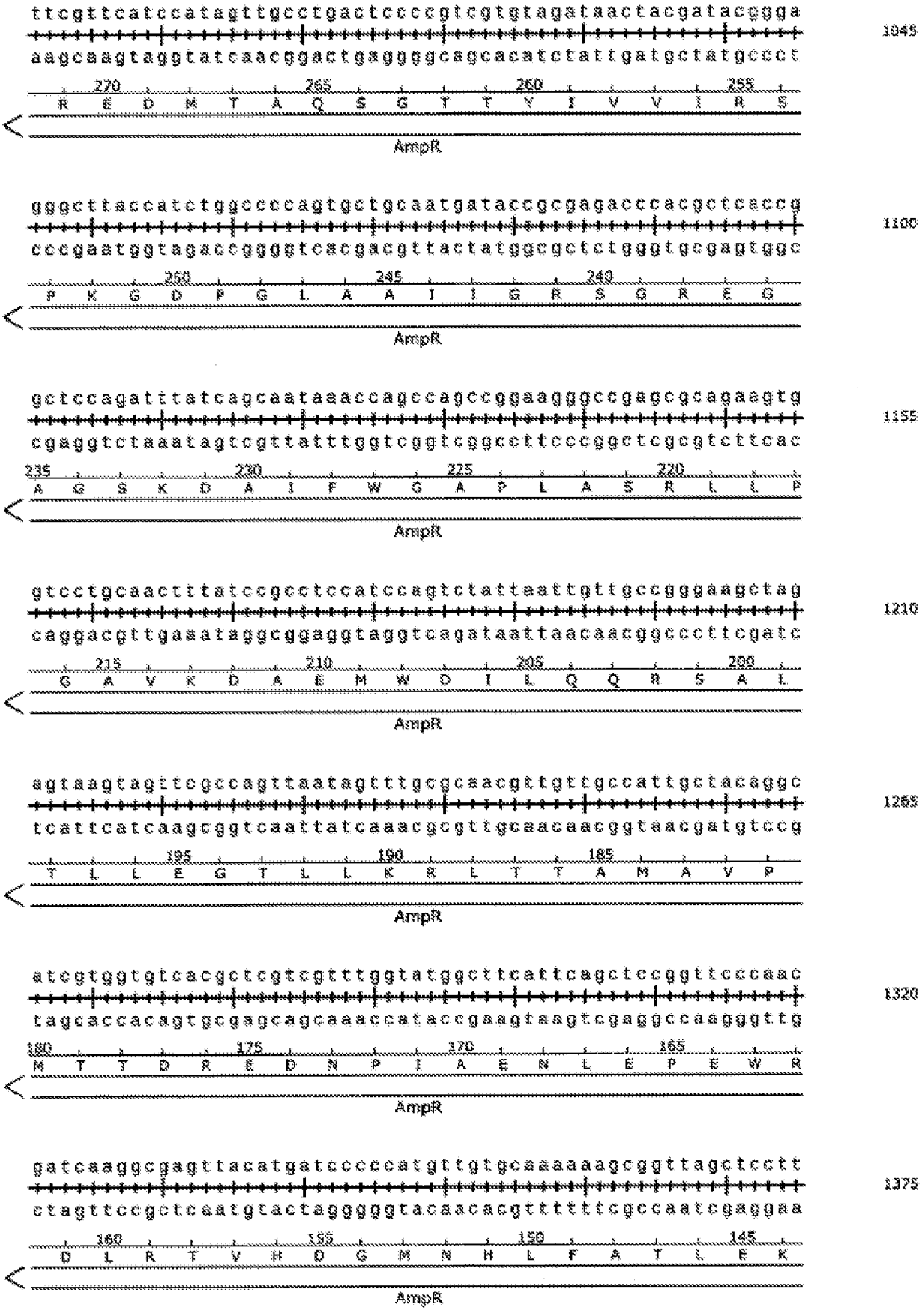


Figure 7 continued

Figure 7 continued



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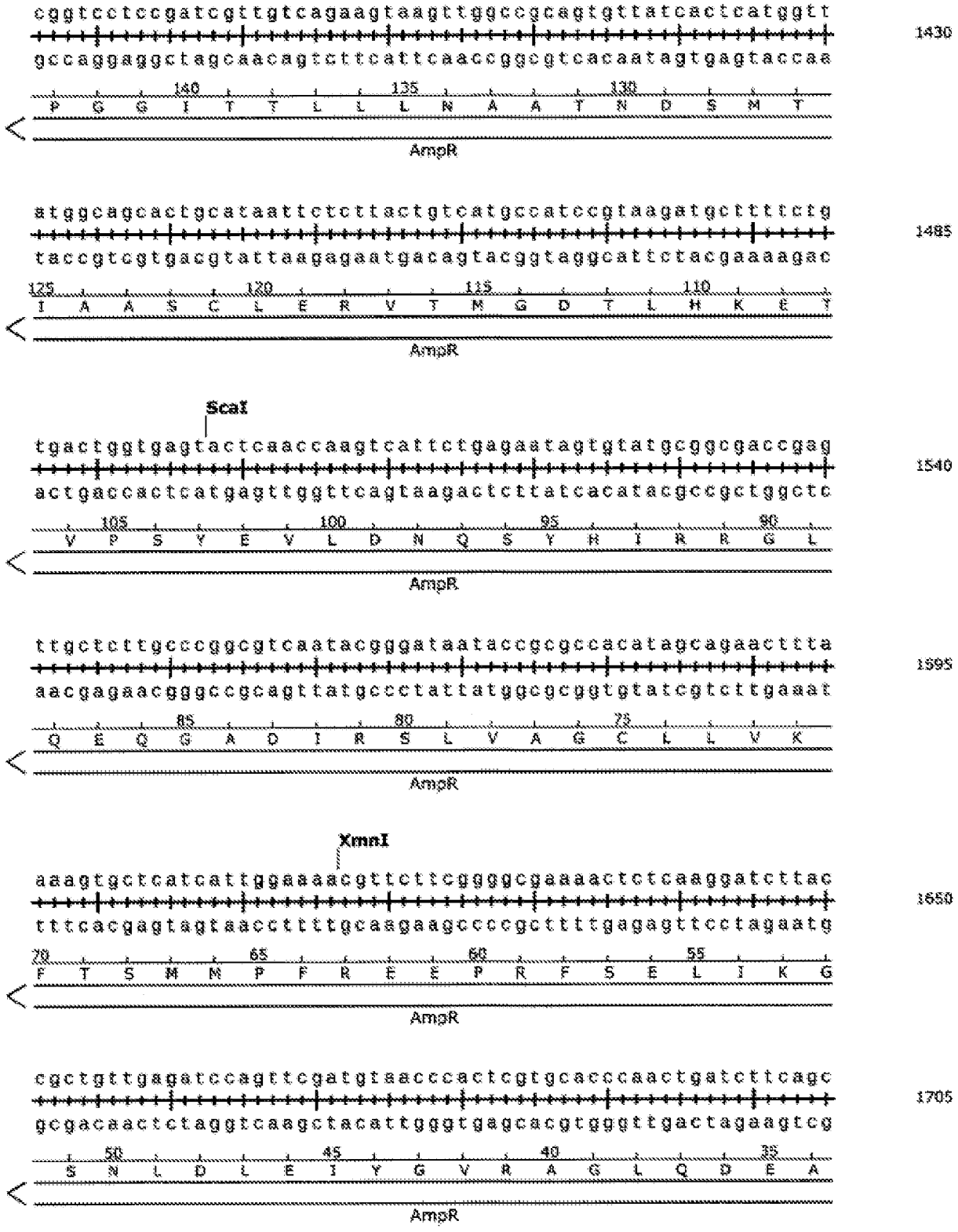

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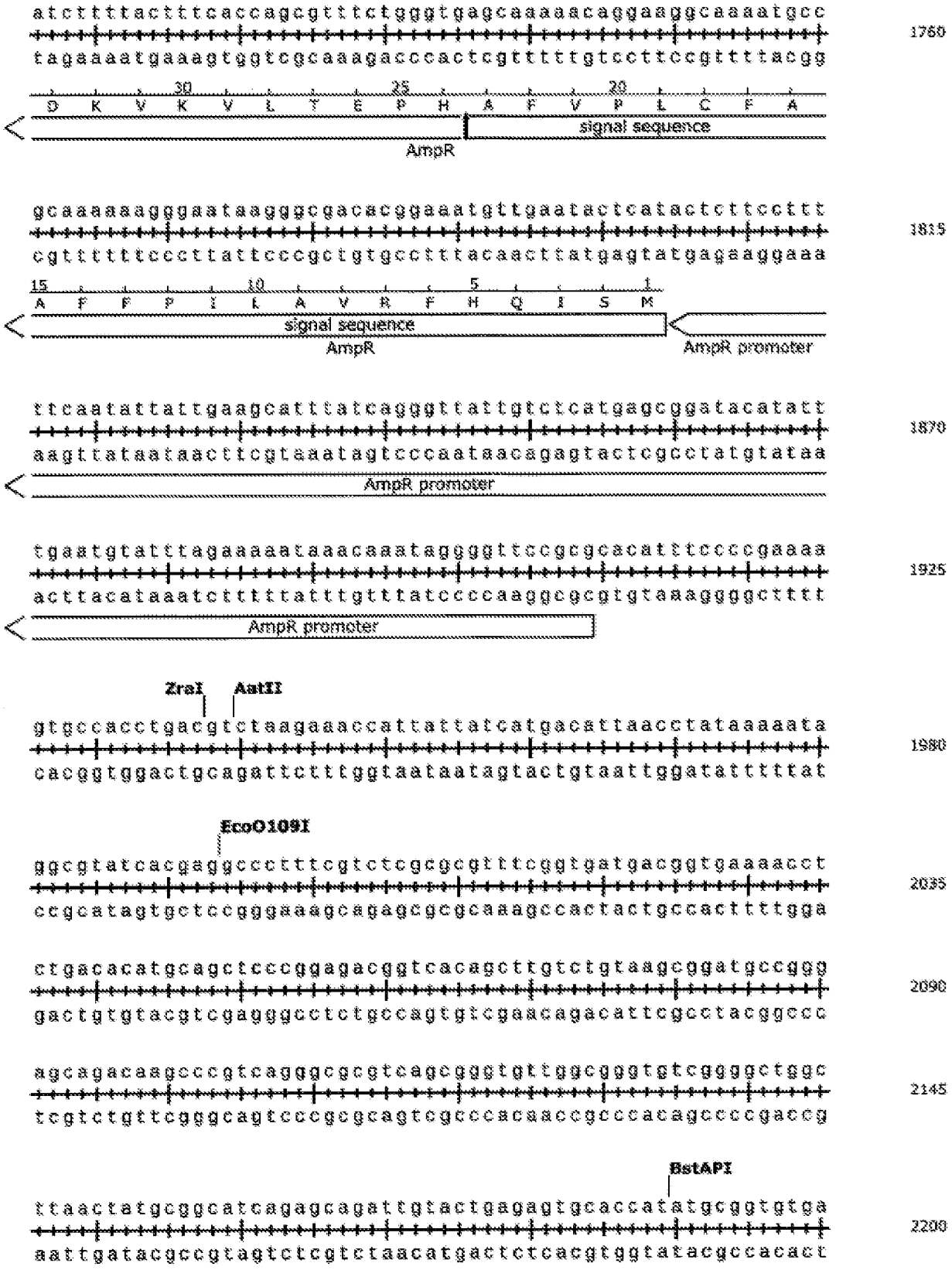

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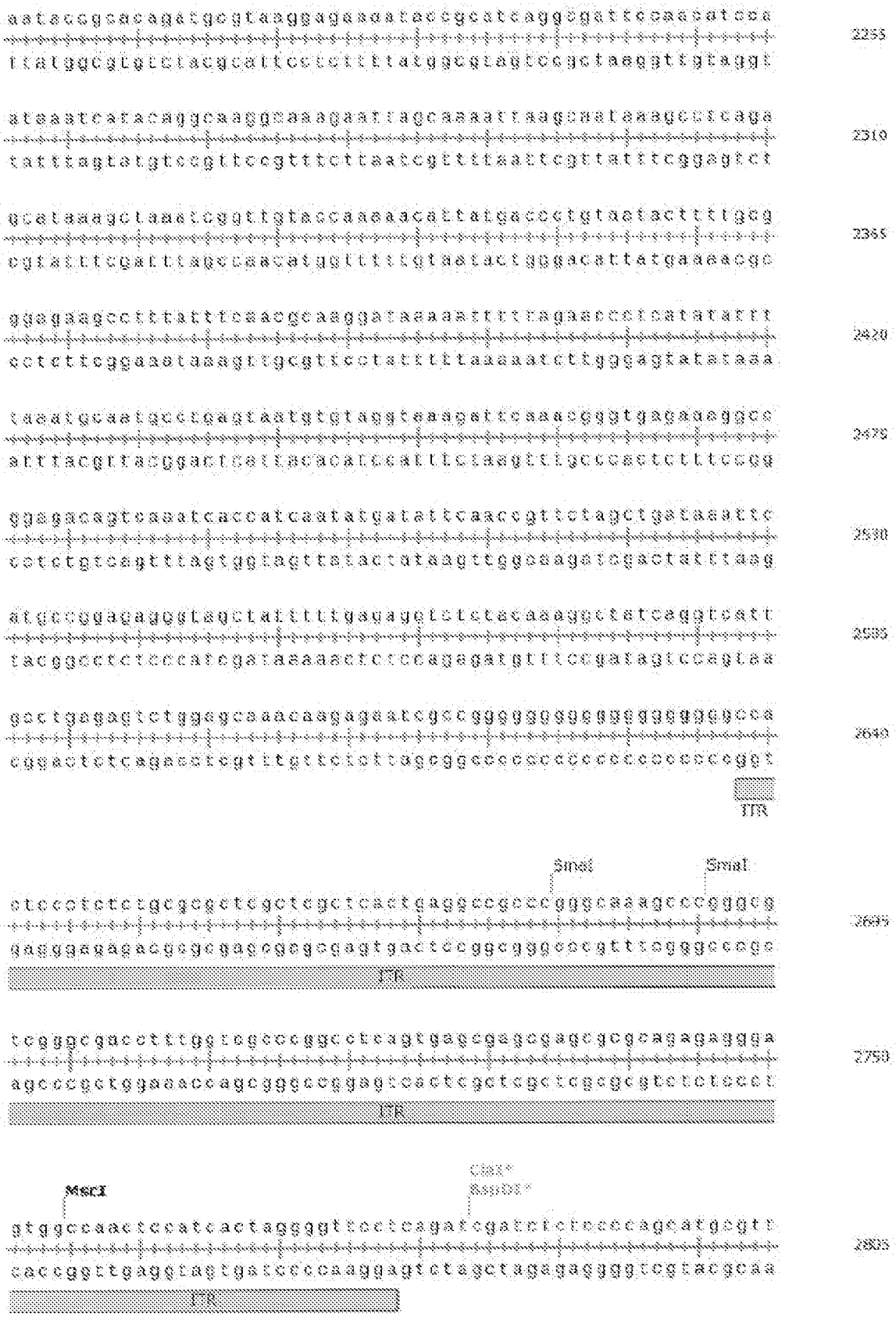

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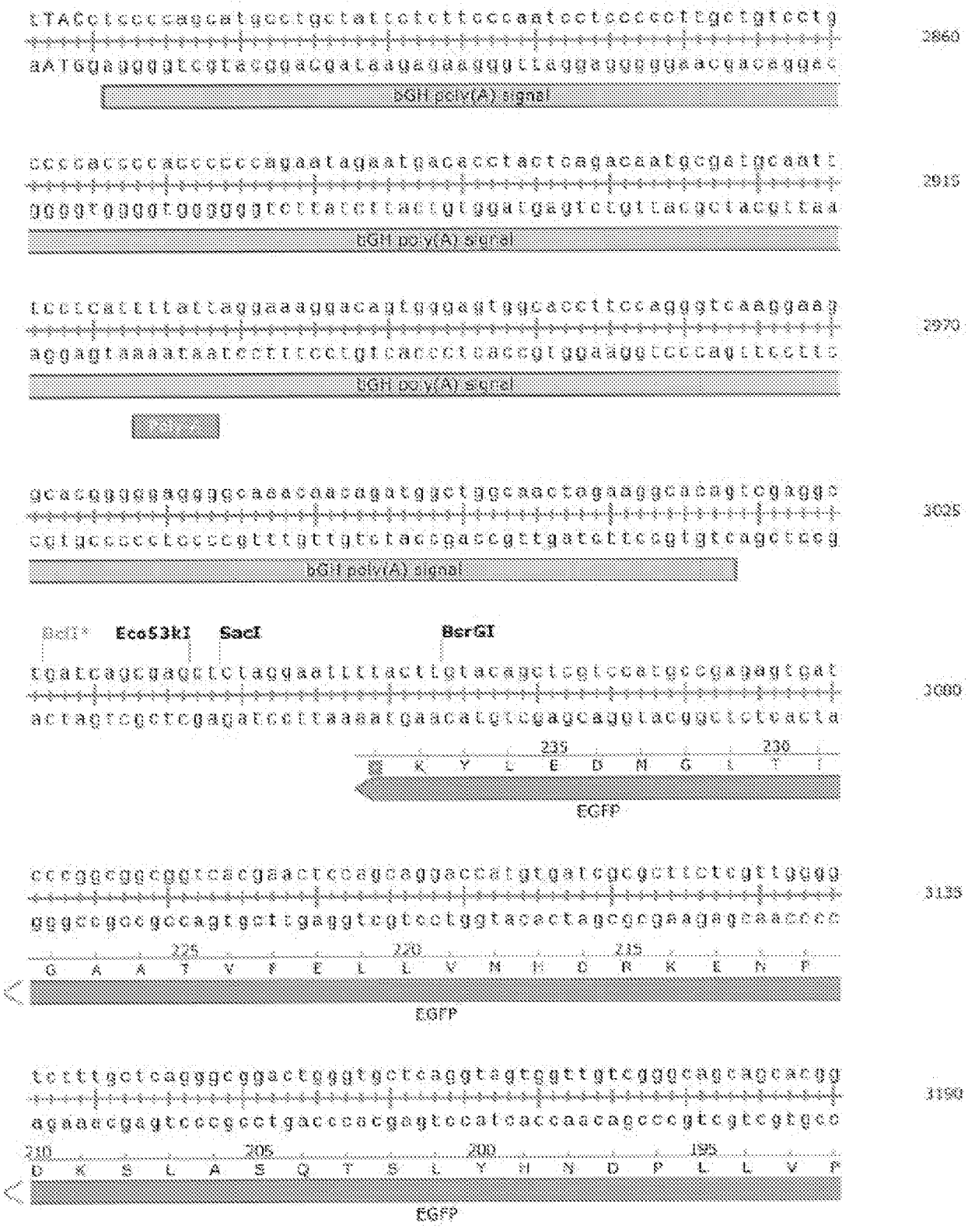

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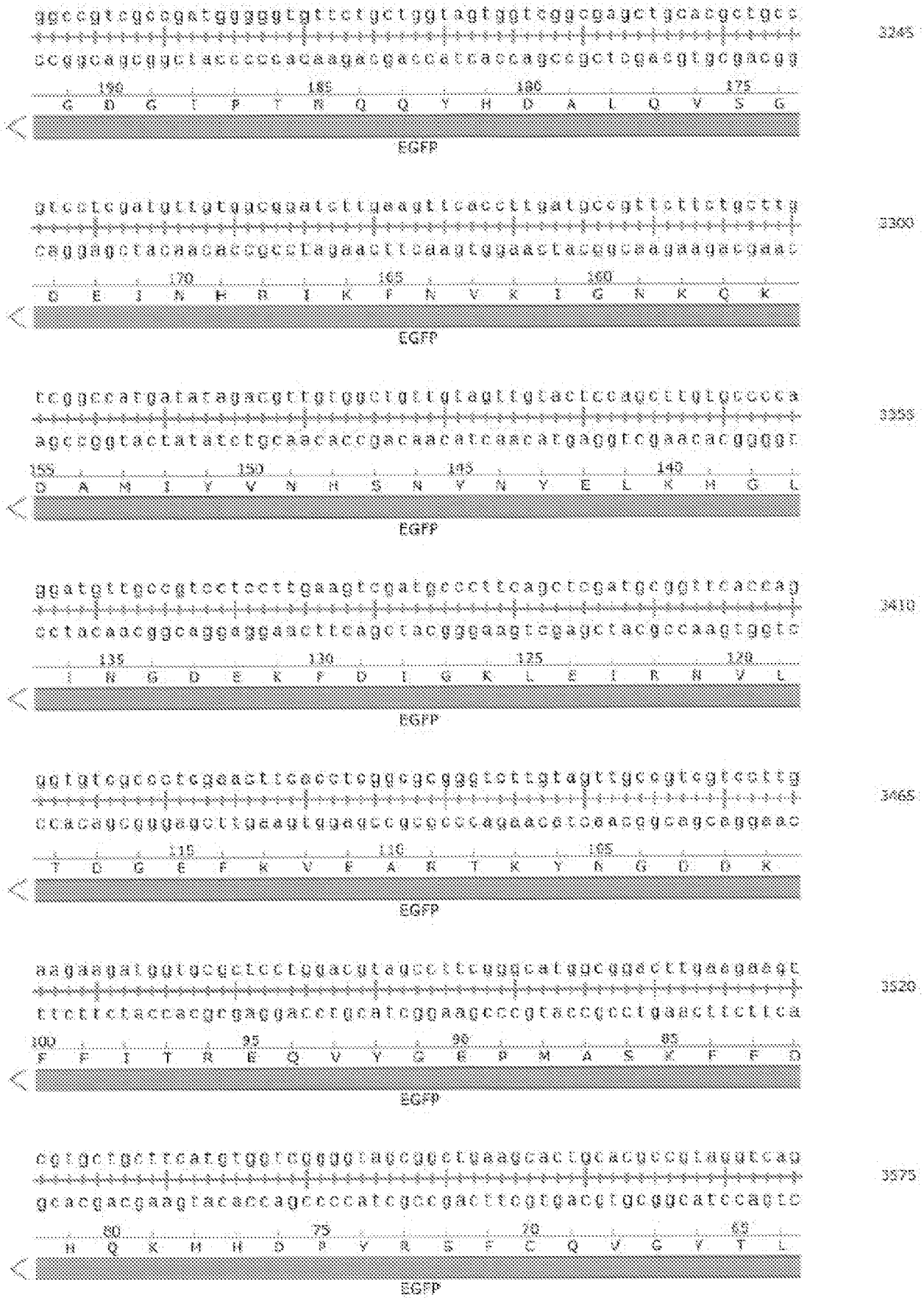

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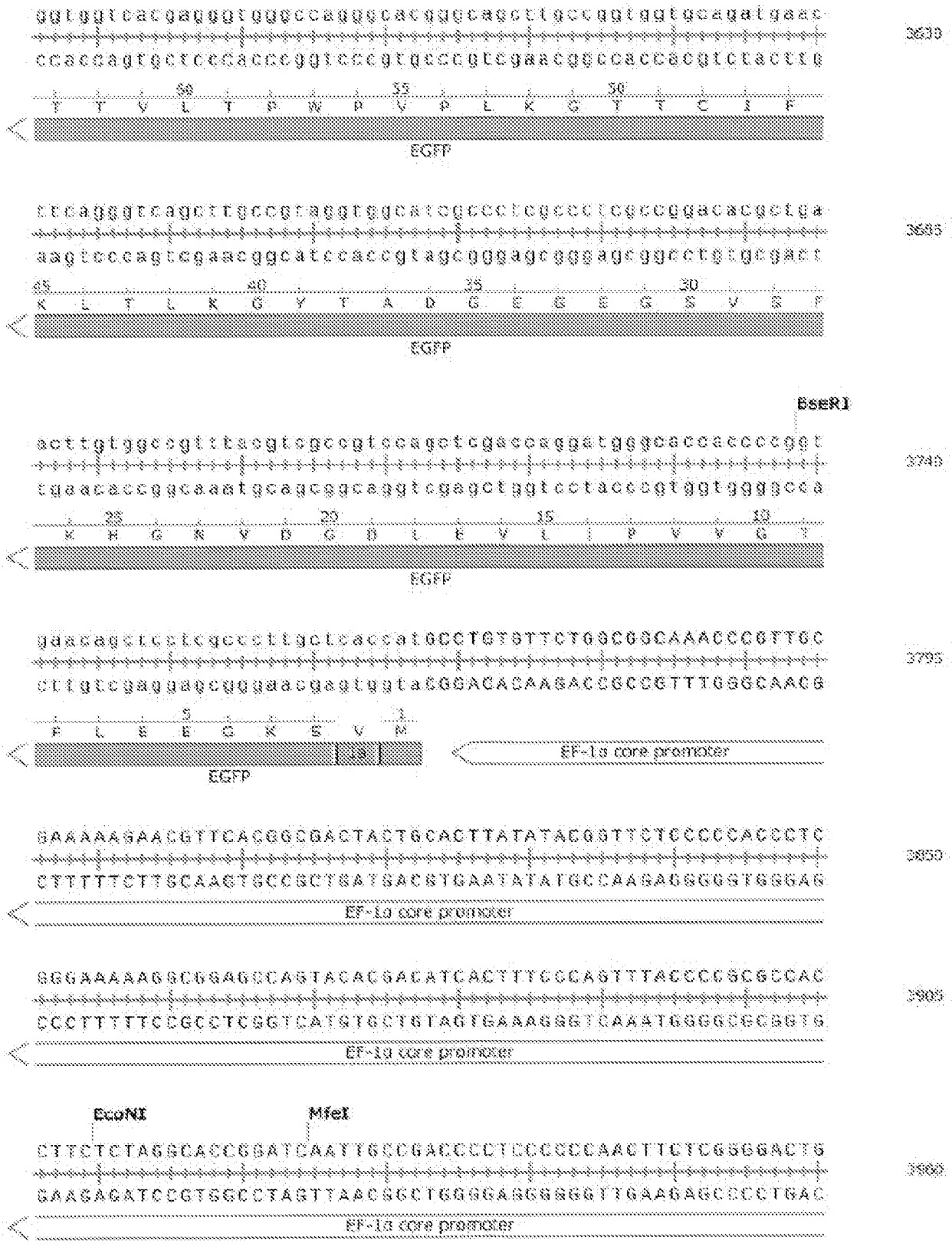

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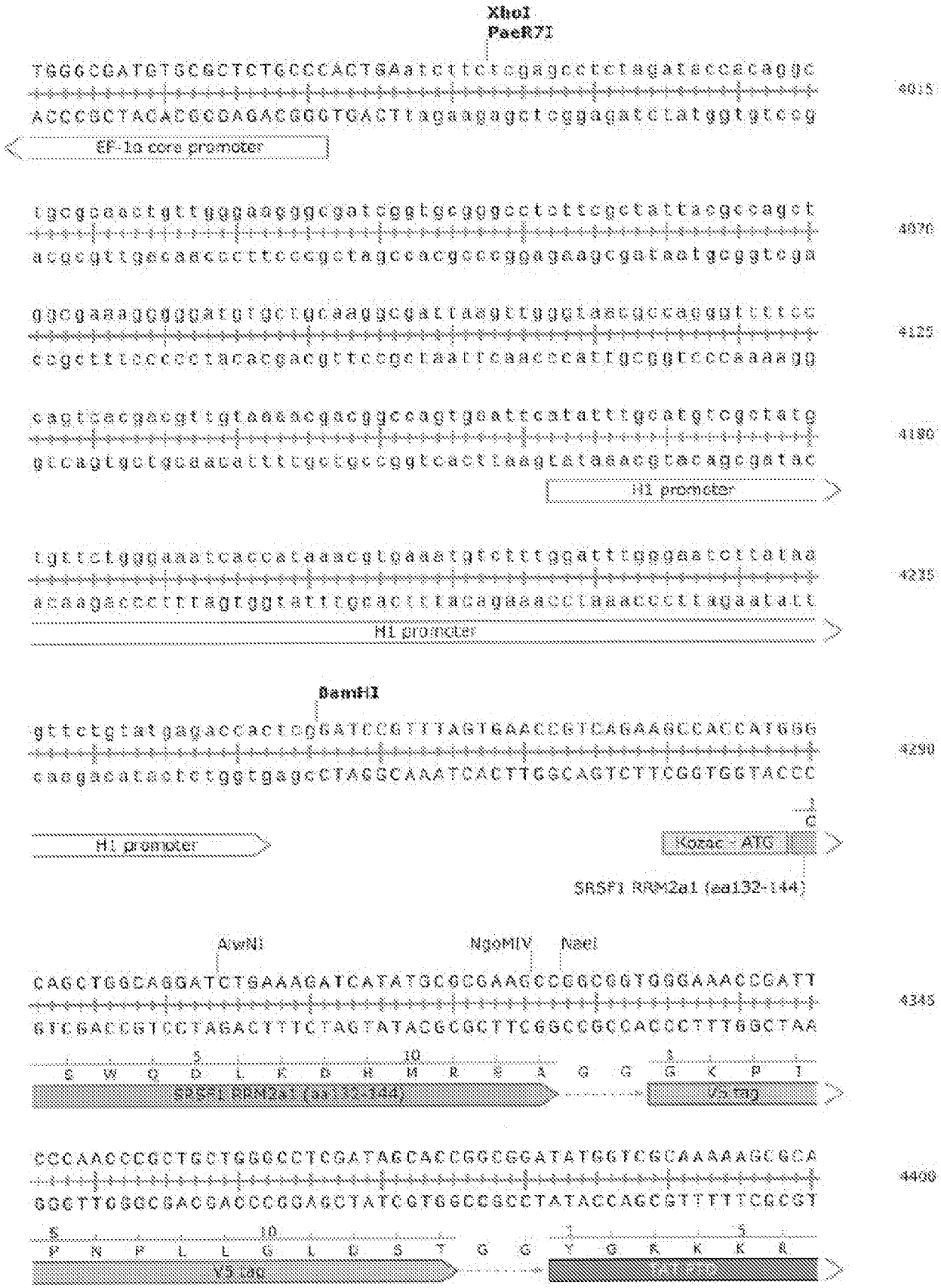

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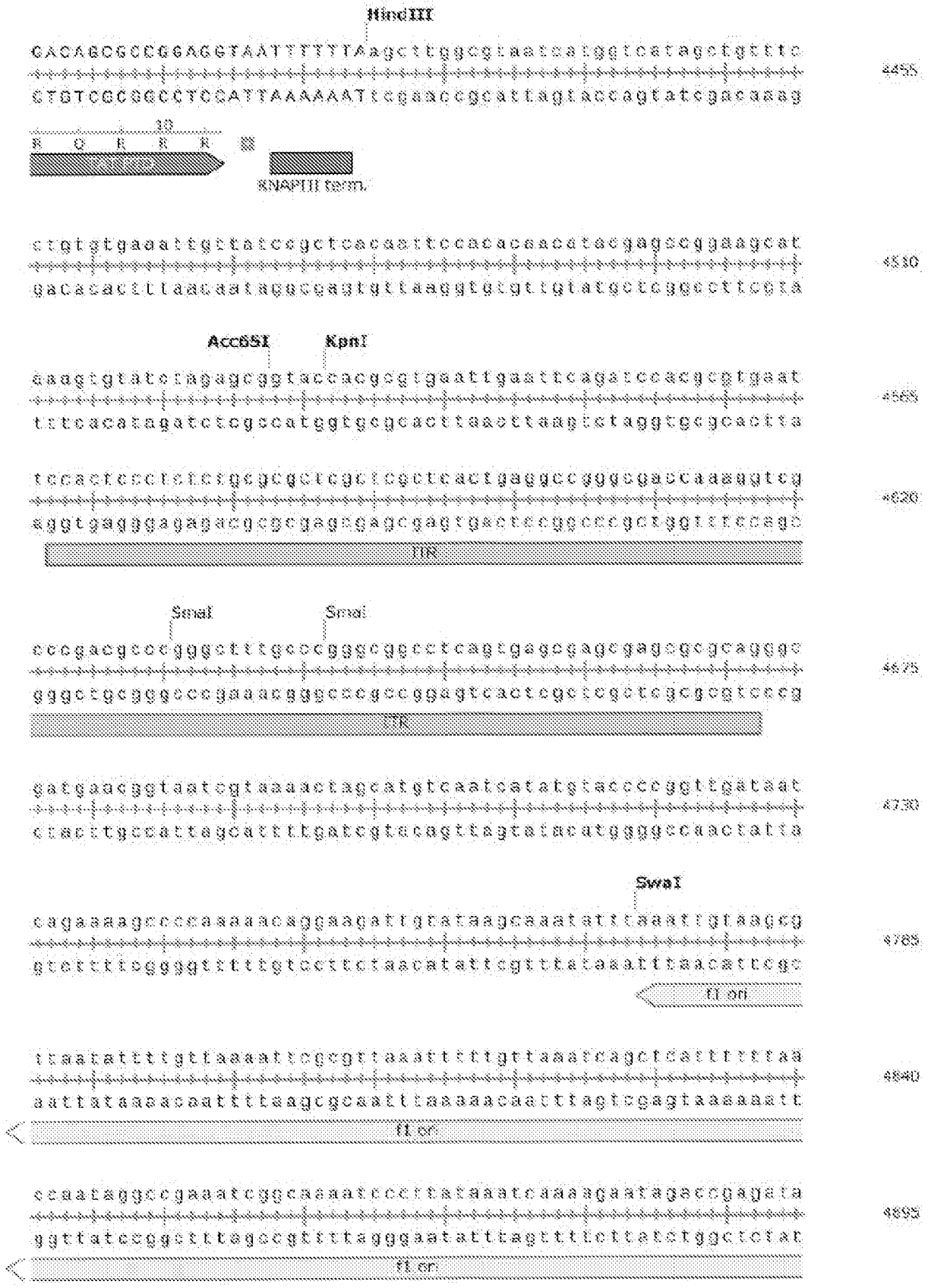


Figure 7 continued

Figure 7 continued

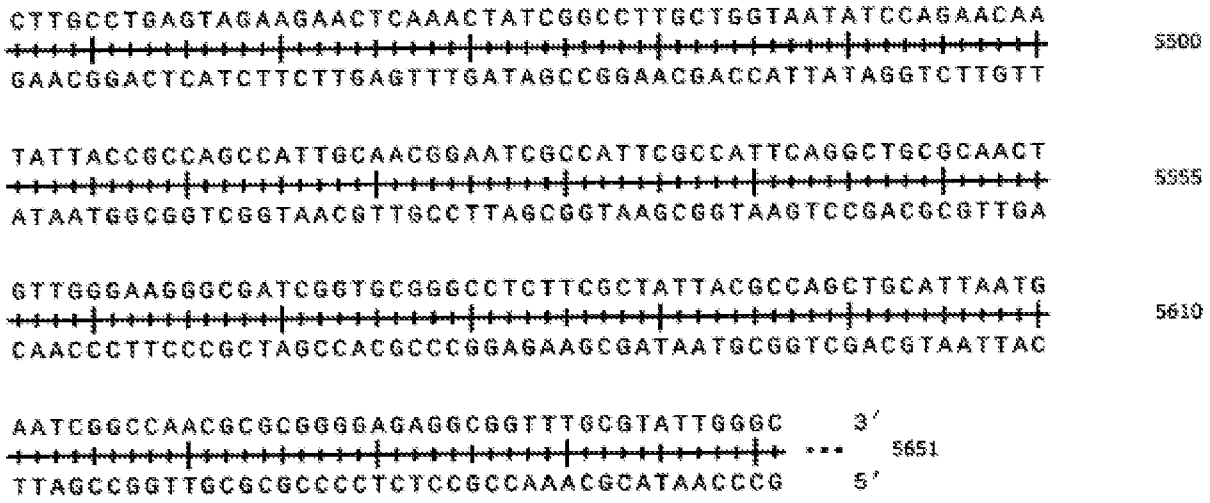


Figure 8

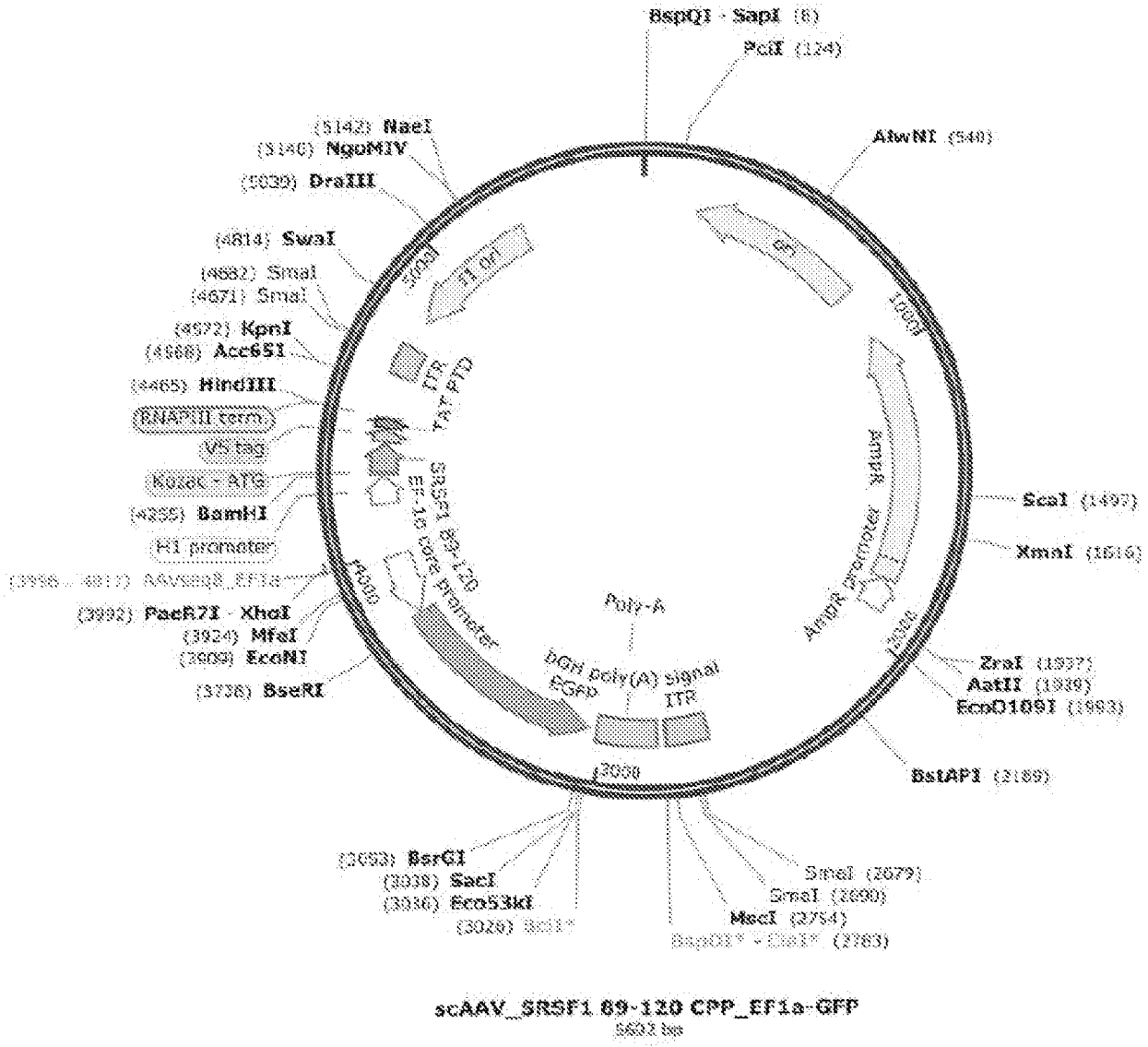

BspQI - SapI (6)
PciI (124)
AlwNI (548)
(5142) NaeI
(5140) NgoMIV
(5030) DraIII
(4814) SwaI
(4682) SmaI
(4671) SmaI
(4572) KpnI
(4566) Acc65I
(4465) HindIII
T1 ori
ITR
TAT PTD
SRSF1 89-120
EF-1α core promoter
GFP
AmpR
AmpR promoter
ScaI (1497)
XmaI (1516)
ZraI (1937)
AatII (1939)
Eco0109I (1983)
BstAPI (2189)
Poly-A
DGH polyA signal
EGFP
ITR
(3093) BsrGI
(3038) SacI
(3036) Eco53kI
SmaI (2679)
SmaI (2690)
MscI (2754)
scAAV_SRSF1 89-120 CPP_EF1a-GFP
5603 bp

Figure 8 continued

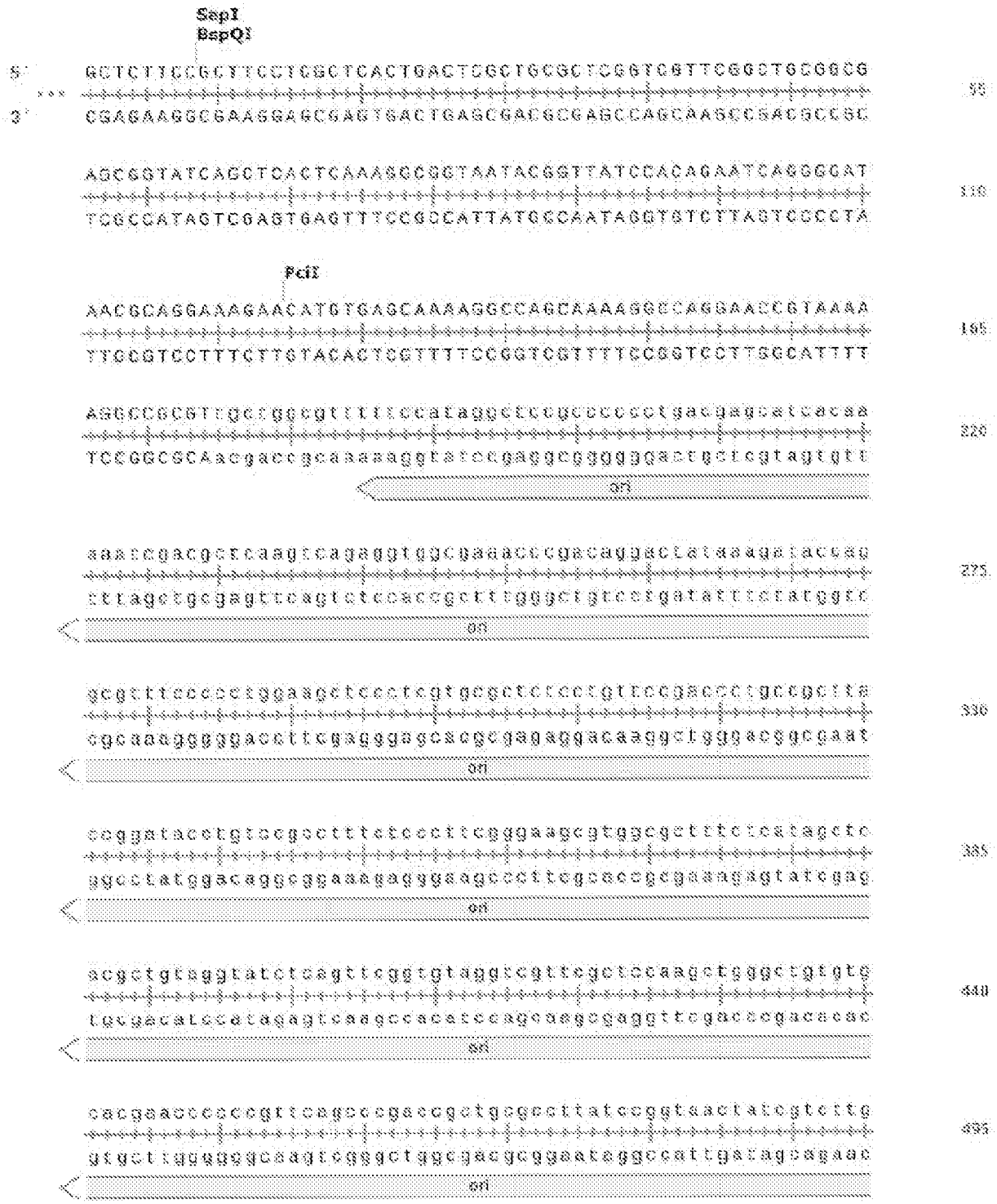
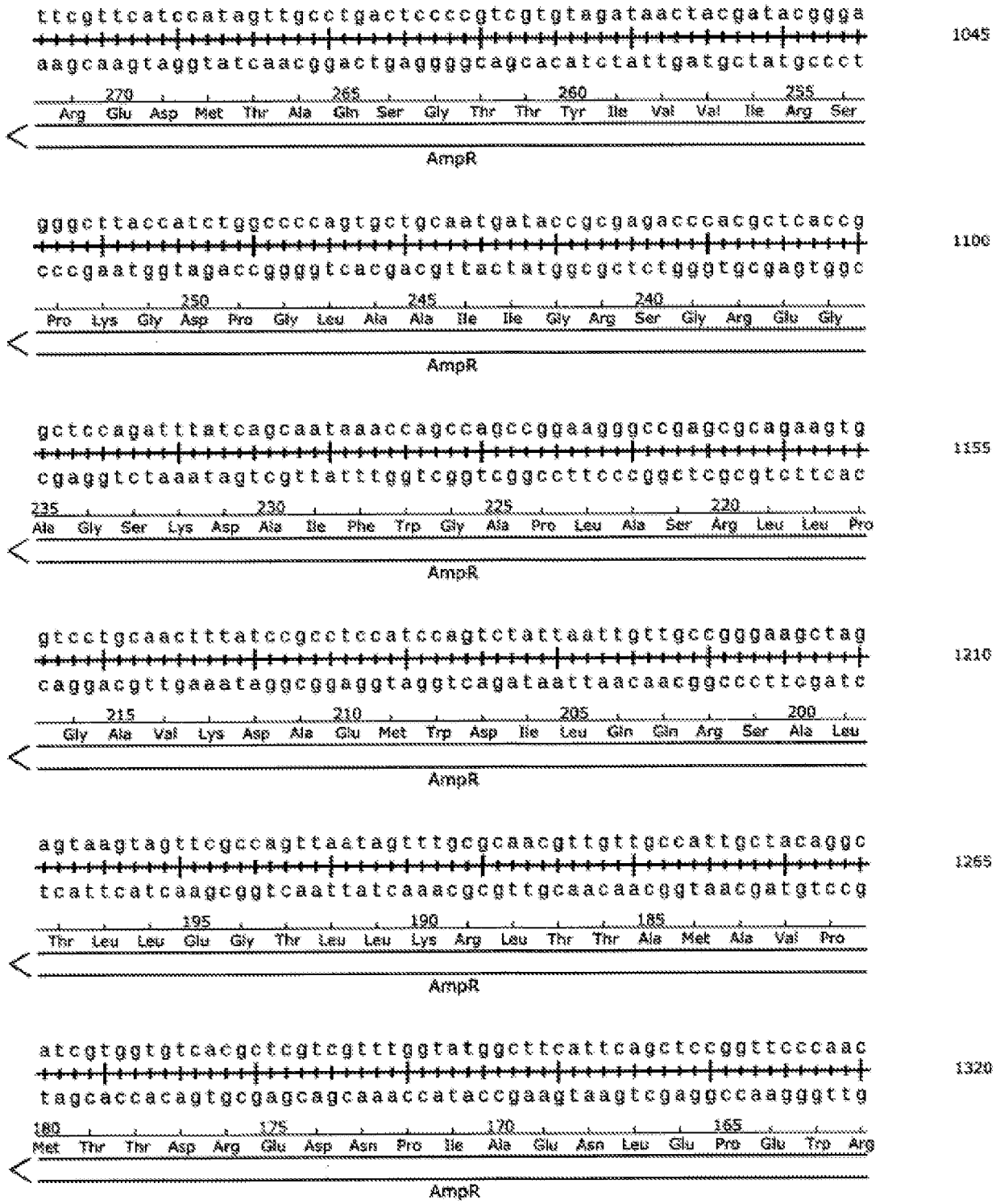


Figure 8 continued

Figure 8 continued



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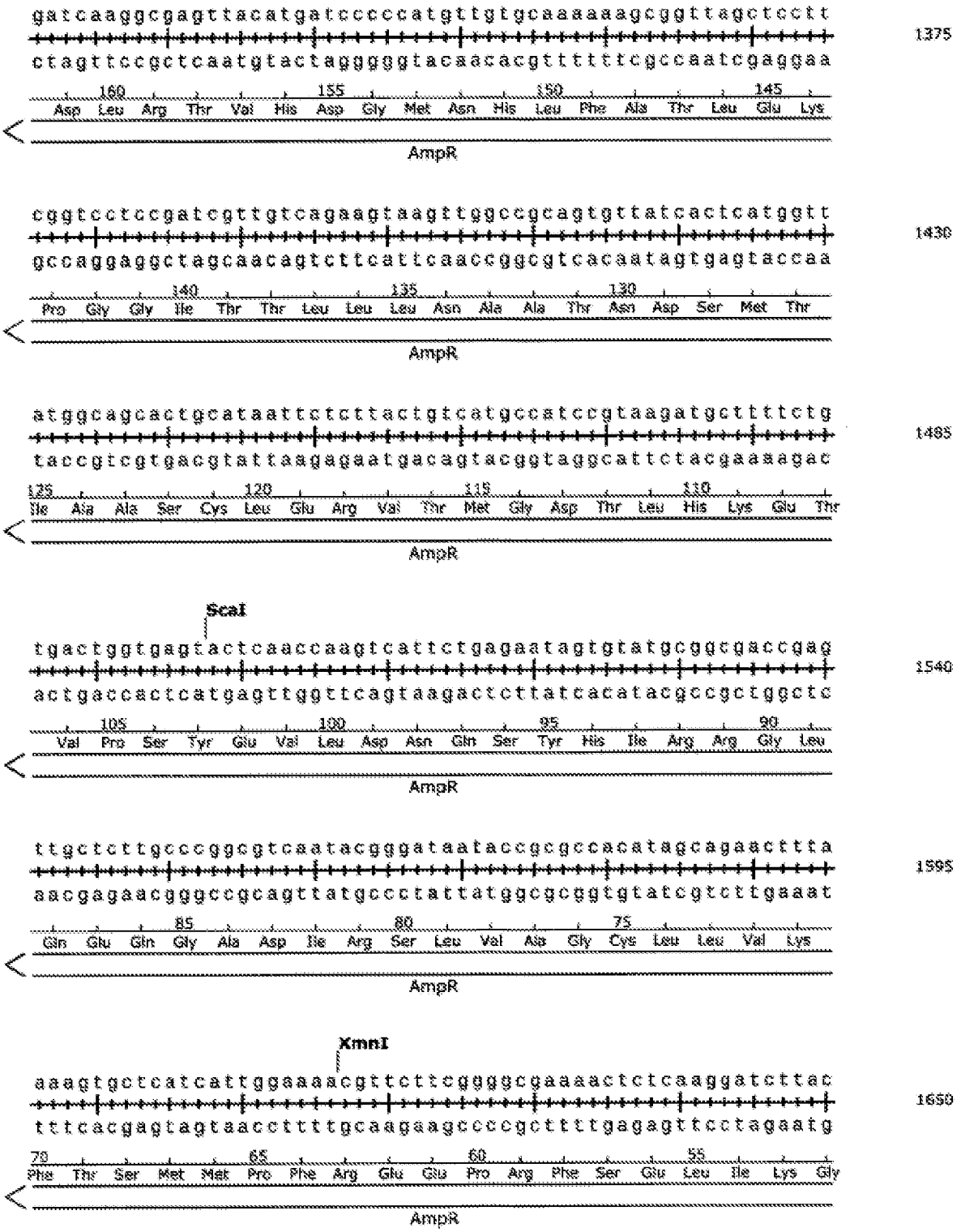

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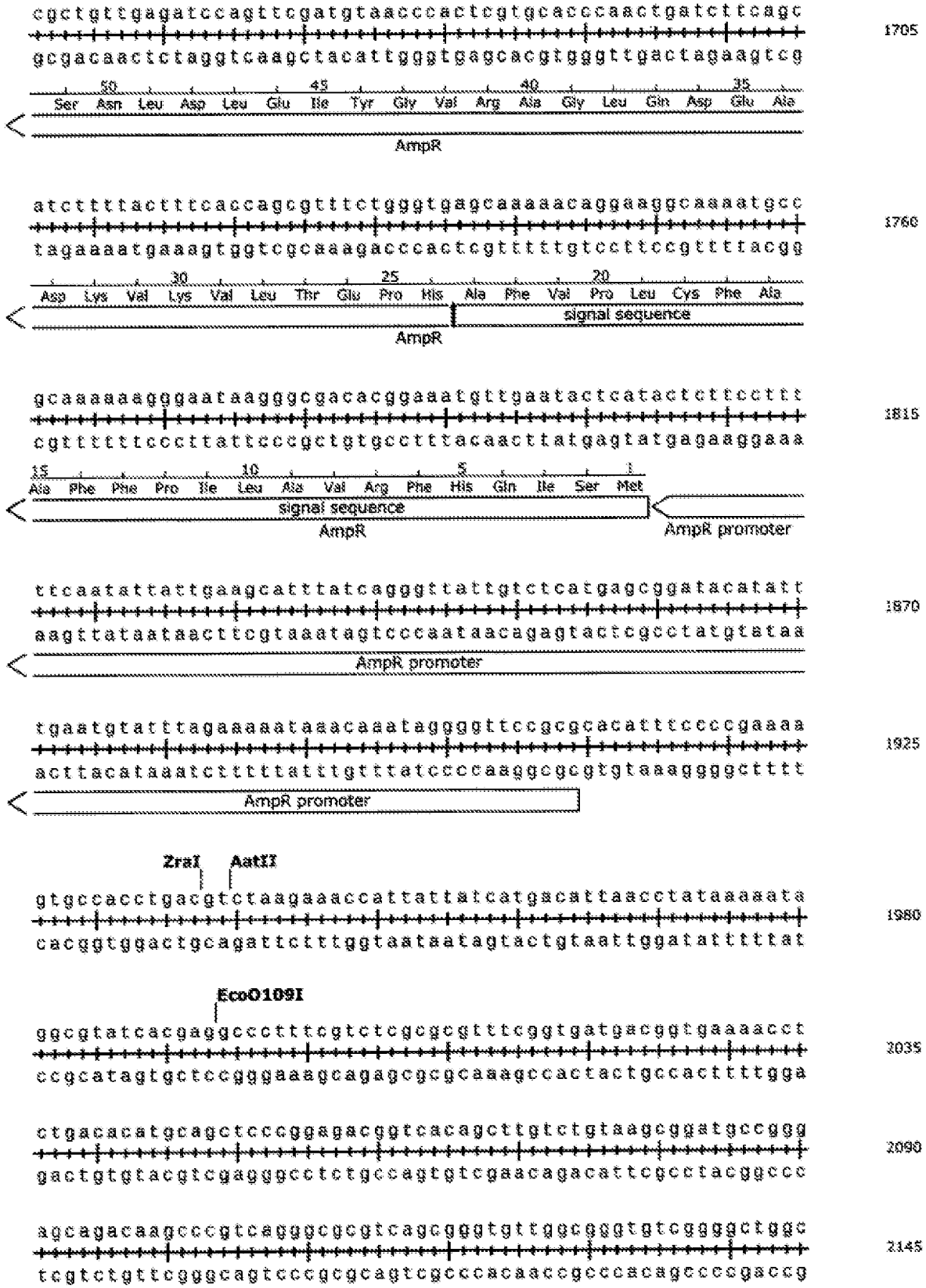
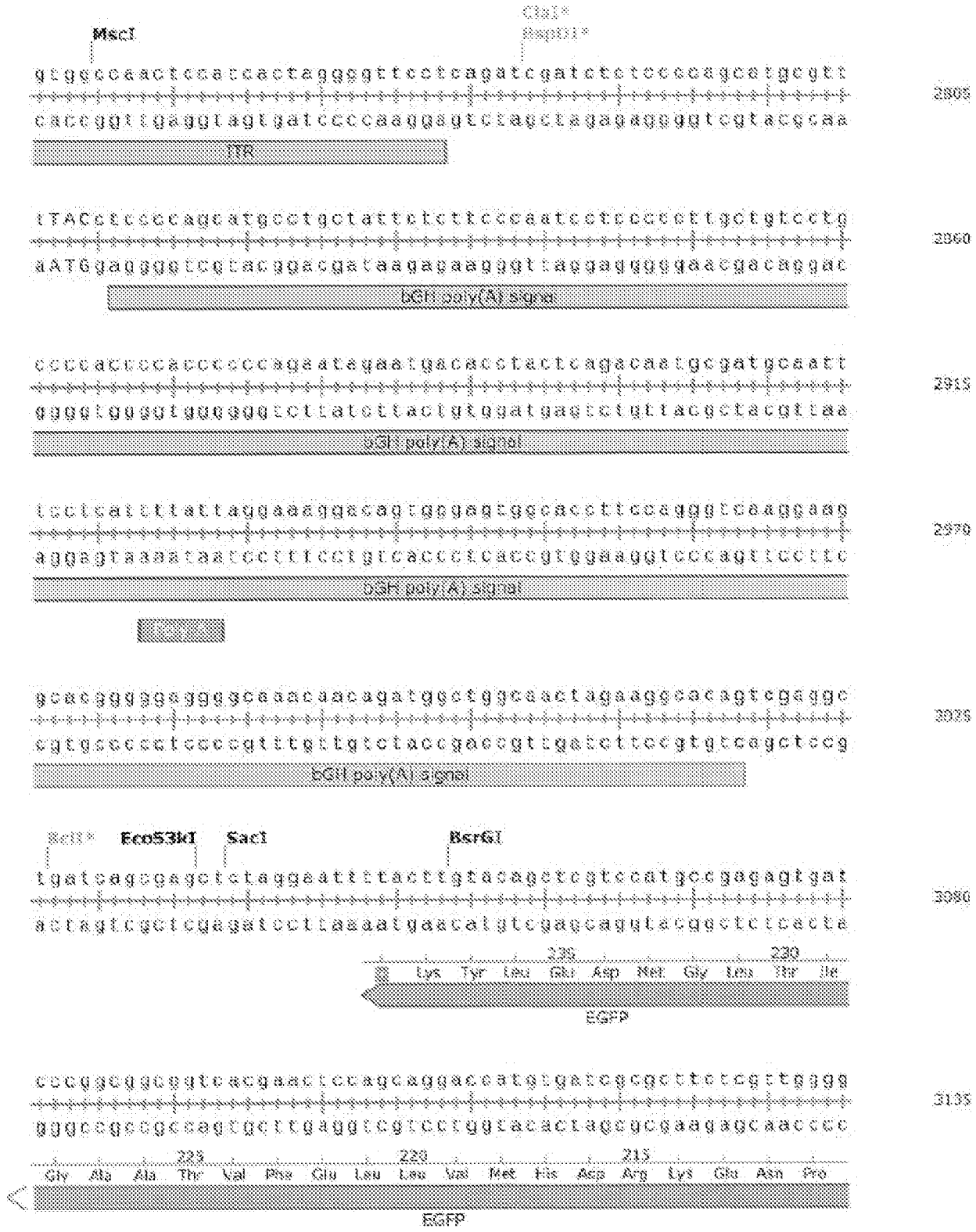


Figure 8 continued

Figure 8 continued



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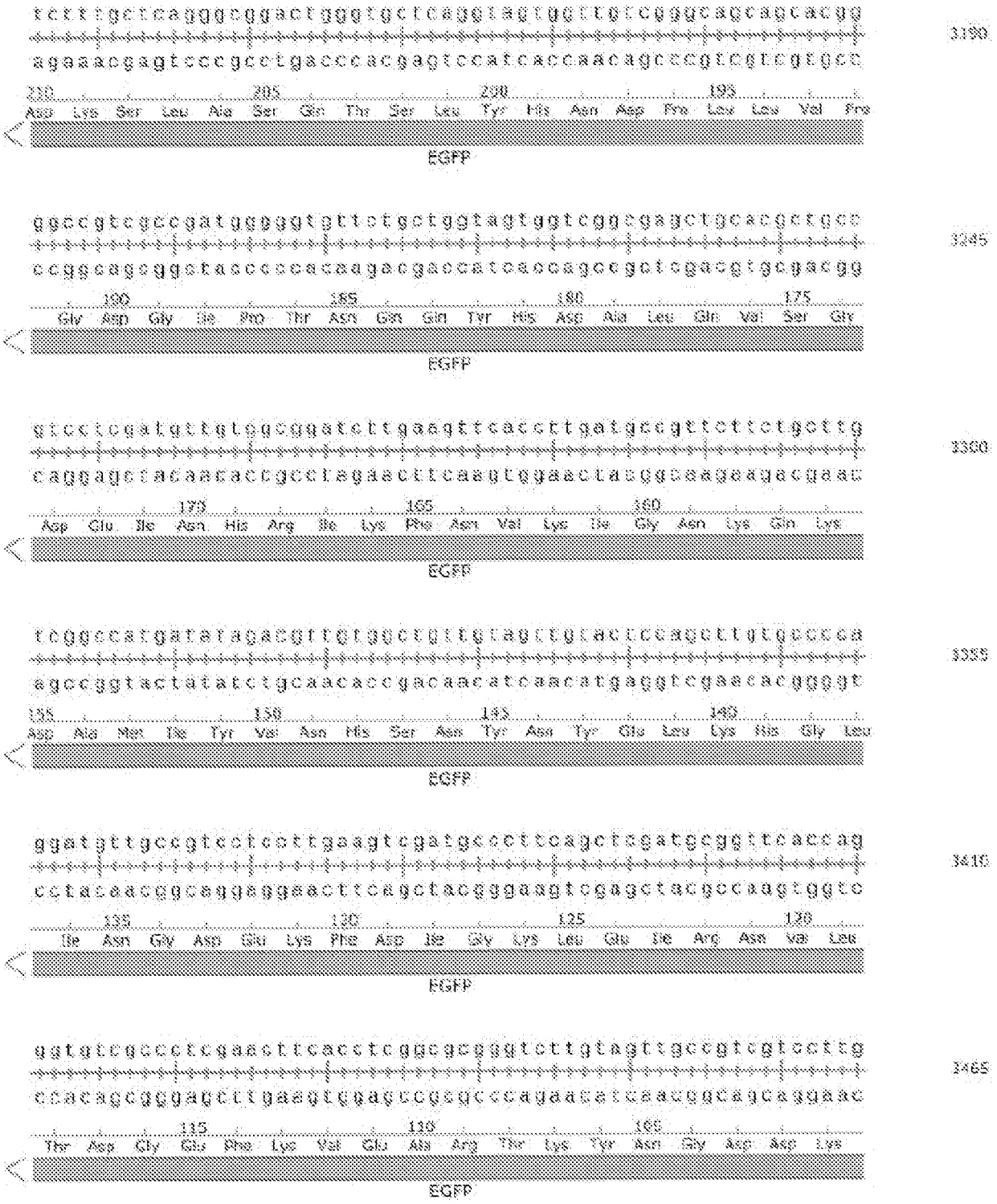

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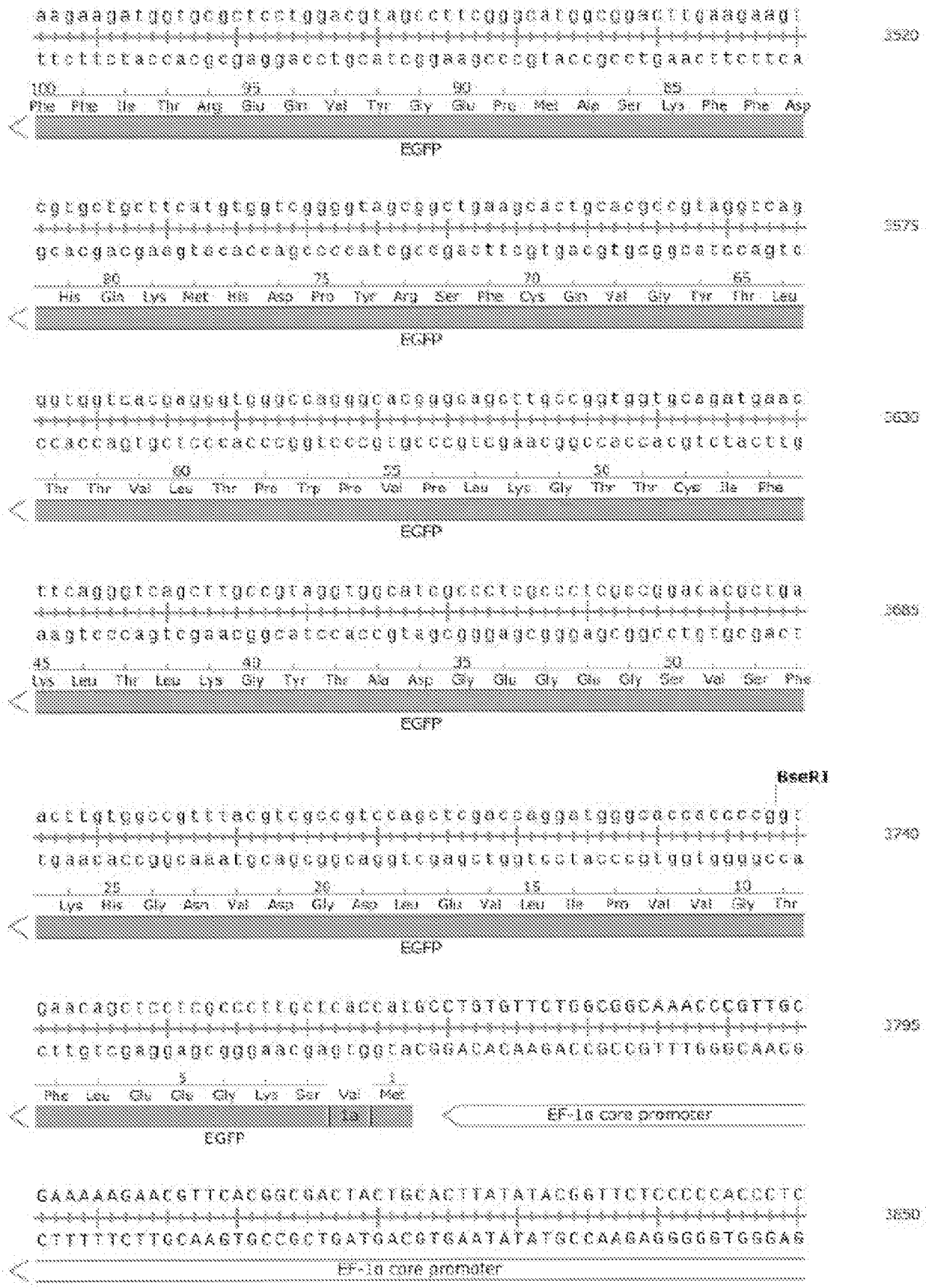

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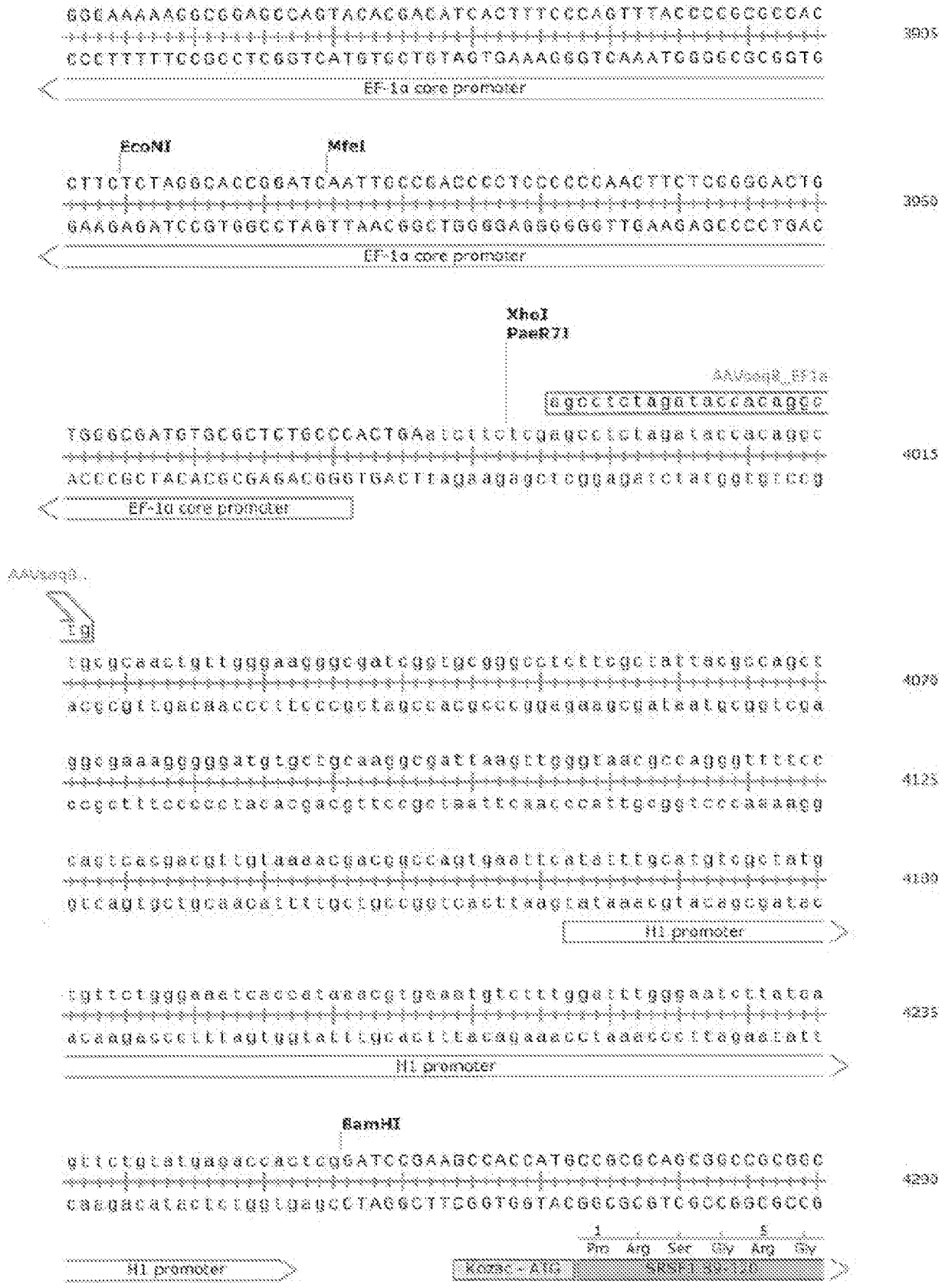

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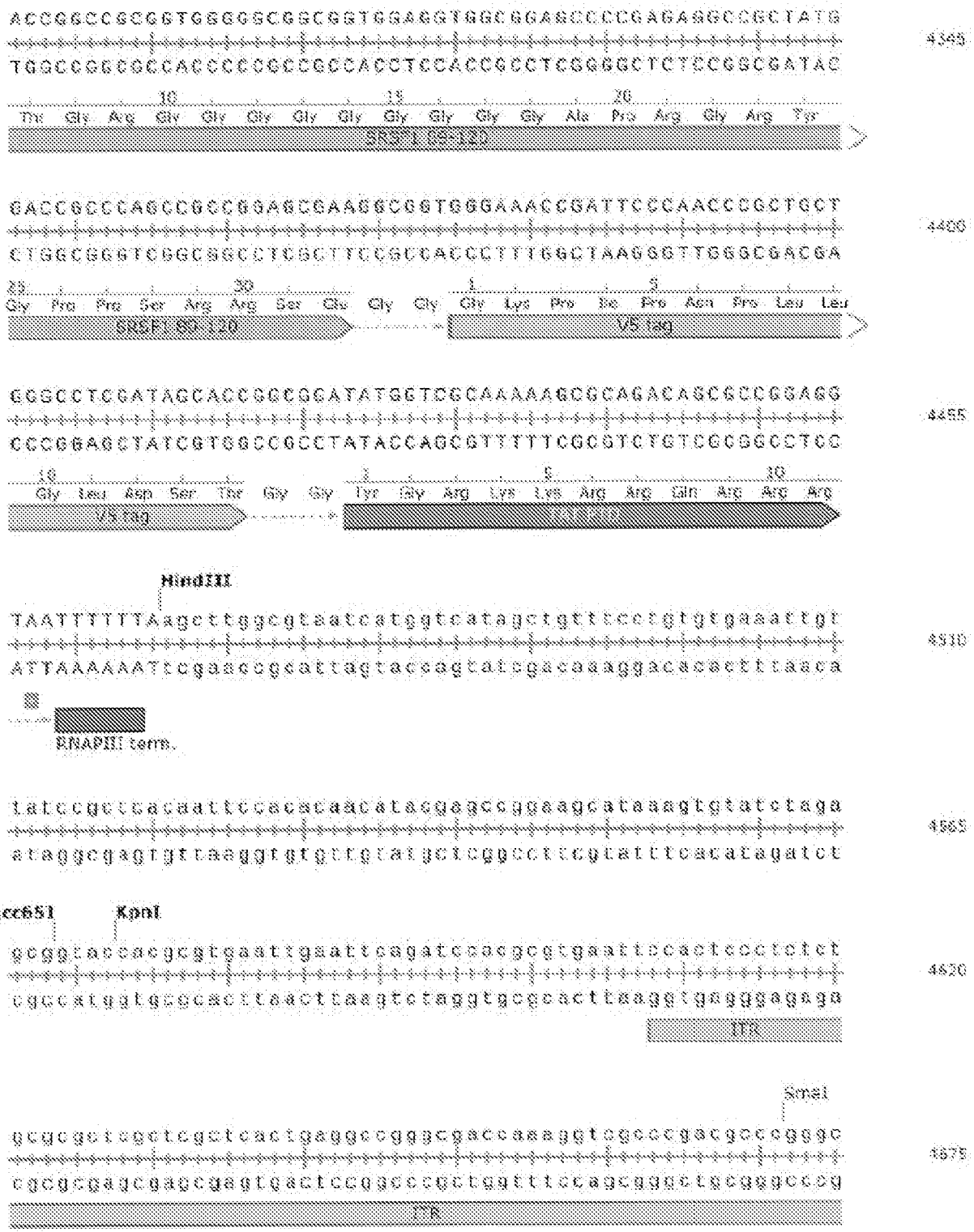

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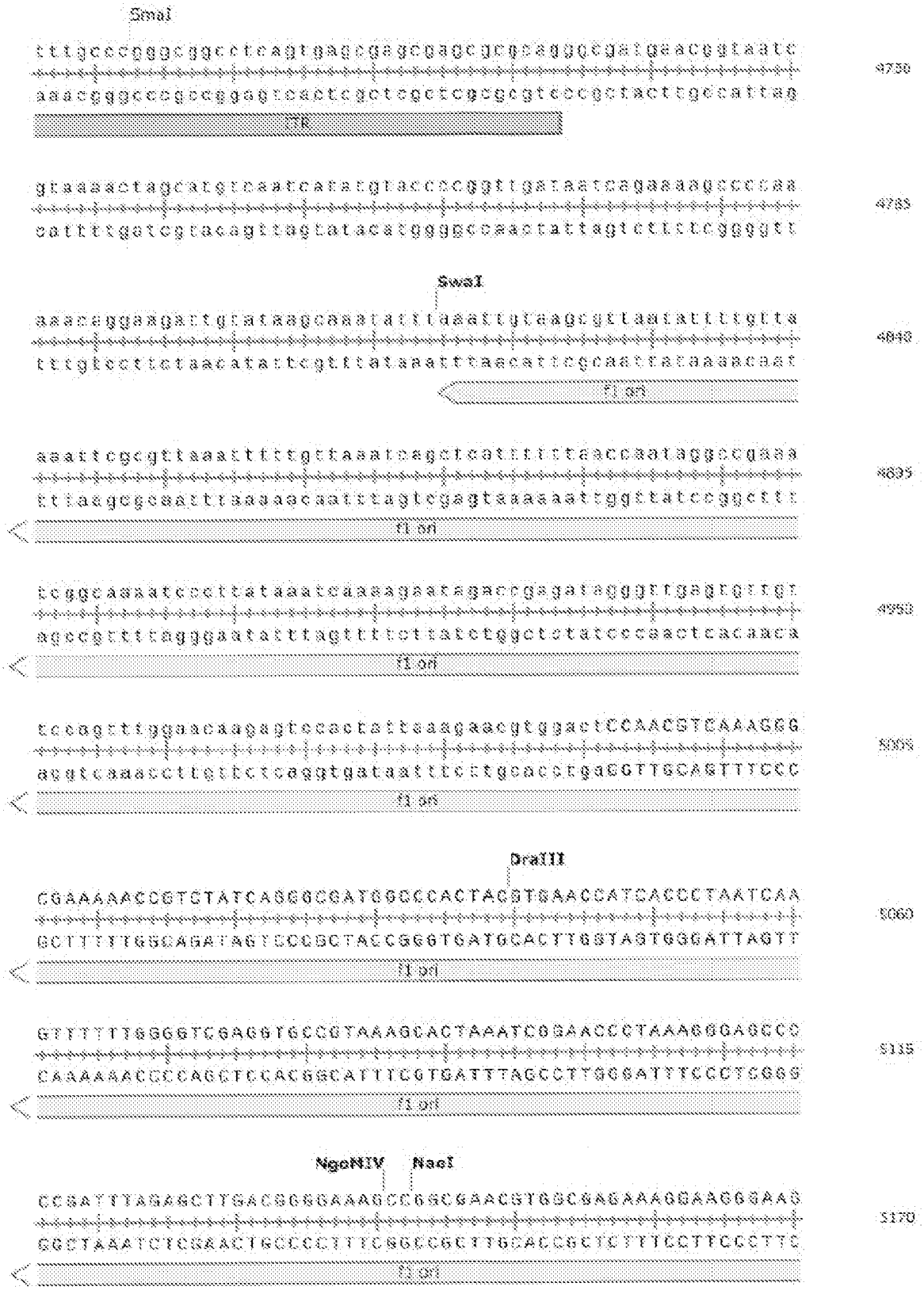

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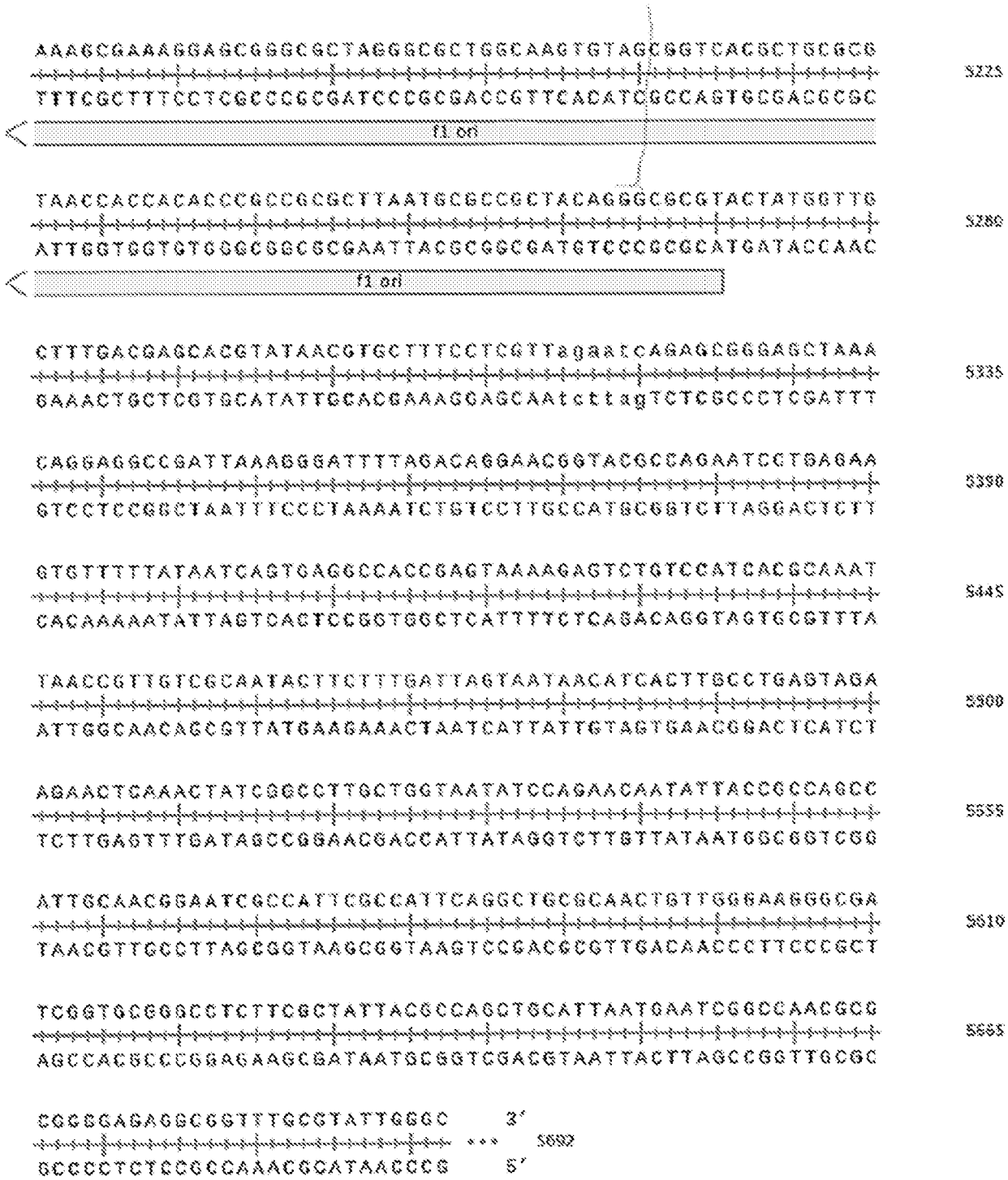


Figure 9

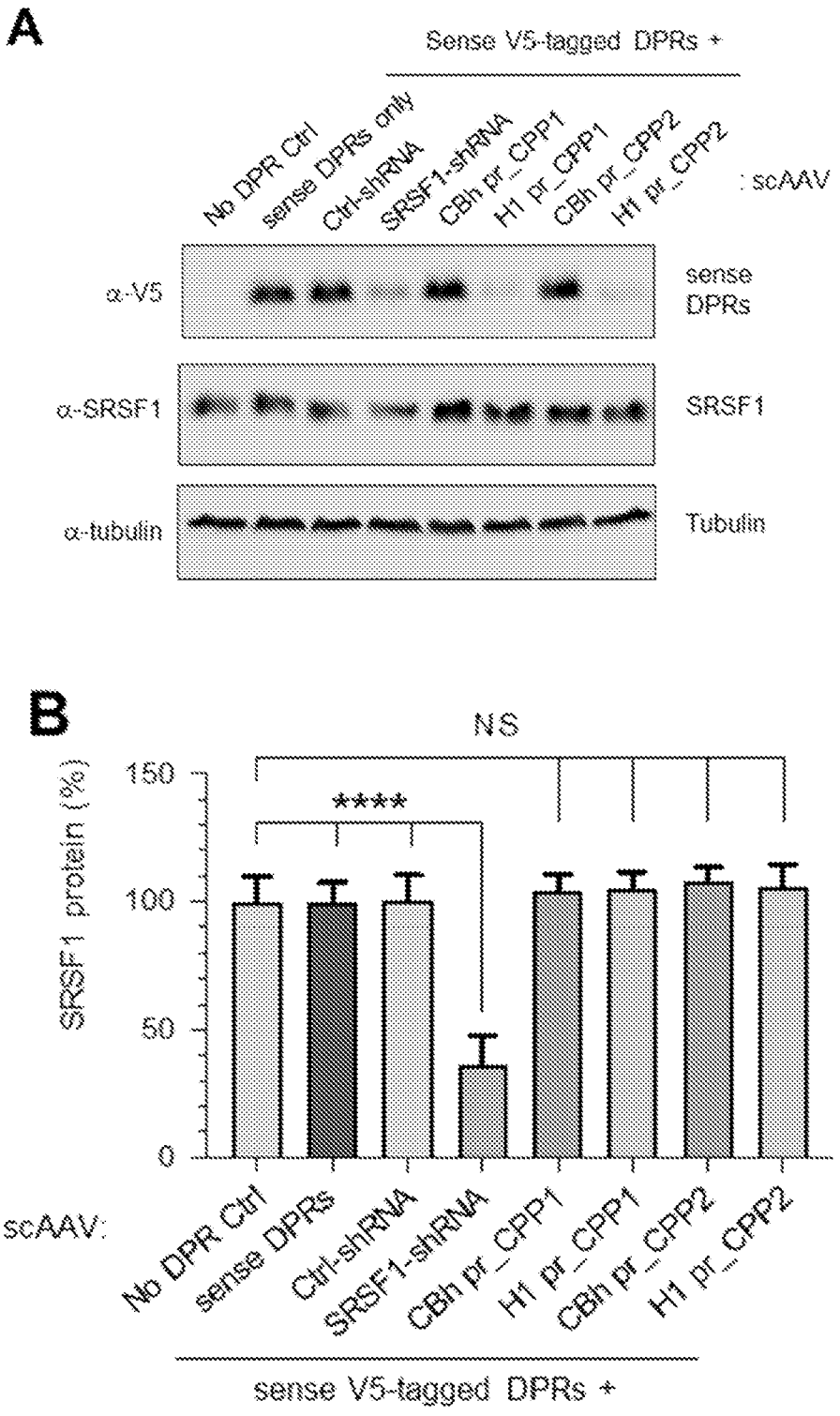
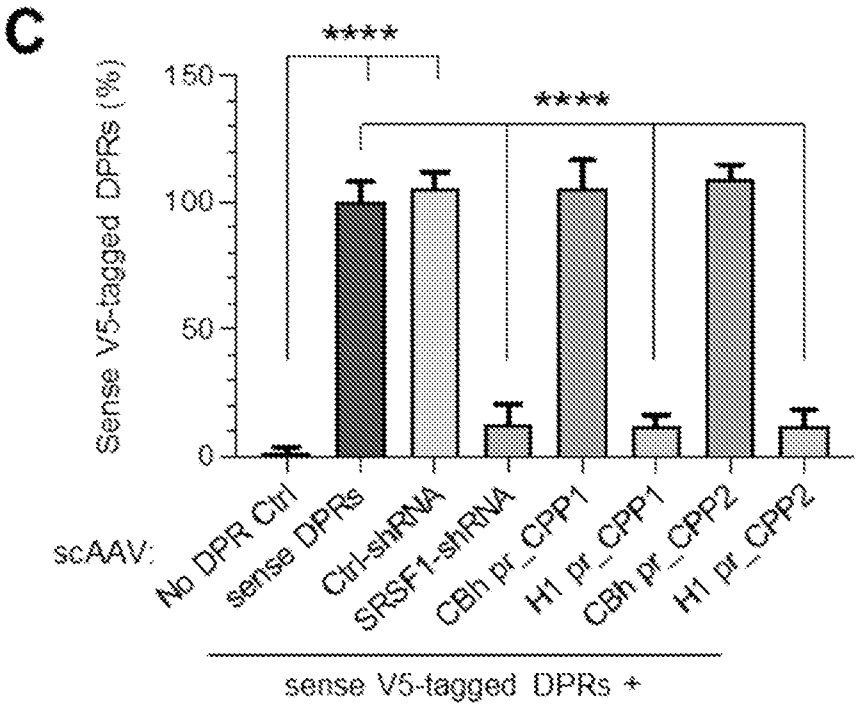

A
Sense V5-tagged DPRs +
No DPR Ctrl
sense DPRs only
Ctrl-shRNA
SRSF1-shRNA
CBh pr_CPP1
H1 pr_CPP1
CBh pr_CPP2
H1 pr_CPP2
: scAAV
α-V5
sense DPRs
α-SRSF1
SRSF1
α-tubulin
Tubulin
B
NS
****
SRSF1 protein (%)
150
100
50
0
scAAV:
No DPR Ctrl
sense DPRs
Ctrl-shRNA
SRSF1-shRNA
CBh pr_CPP1
H1 pr_CPP1
CBh pr_CPP2
H1 pr_CPP2
sense V5-tagged DPRs +

Figure 9 continued



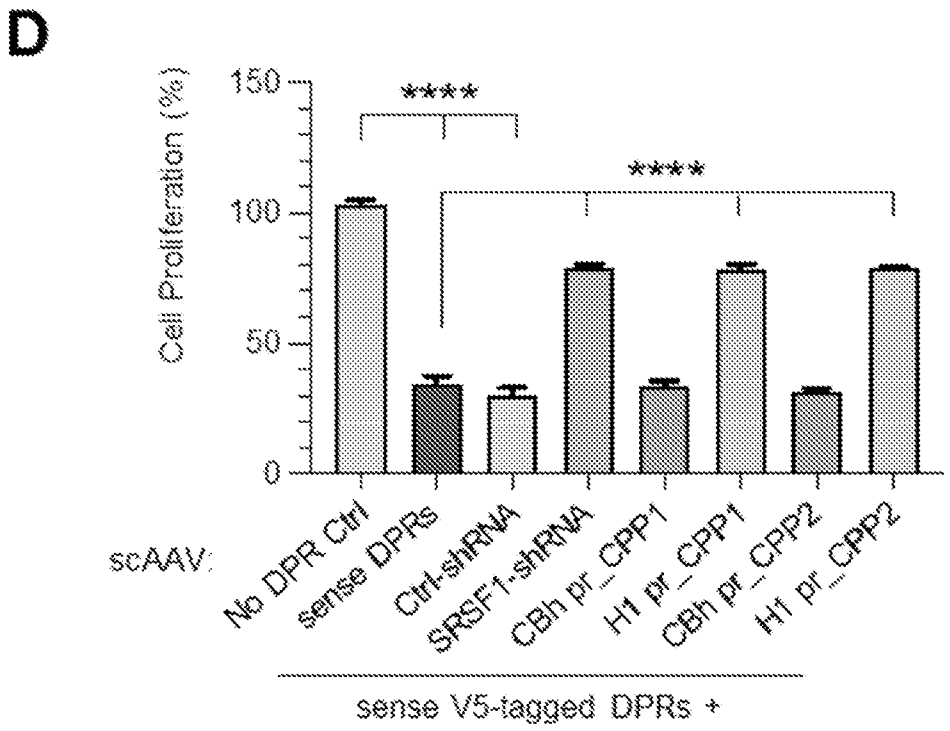


Figure 10

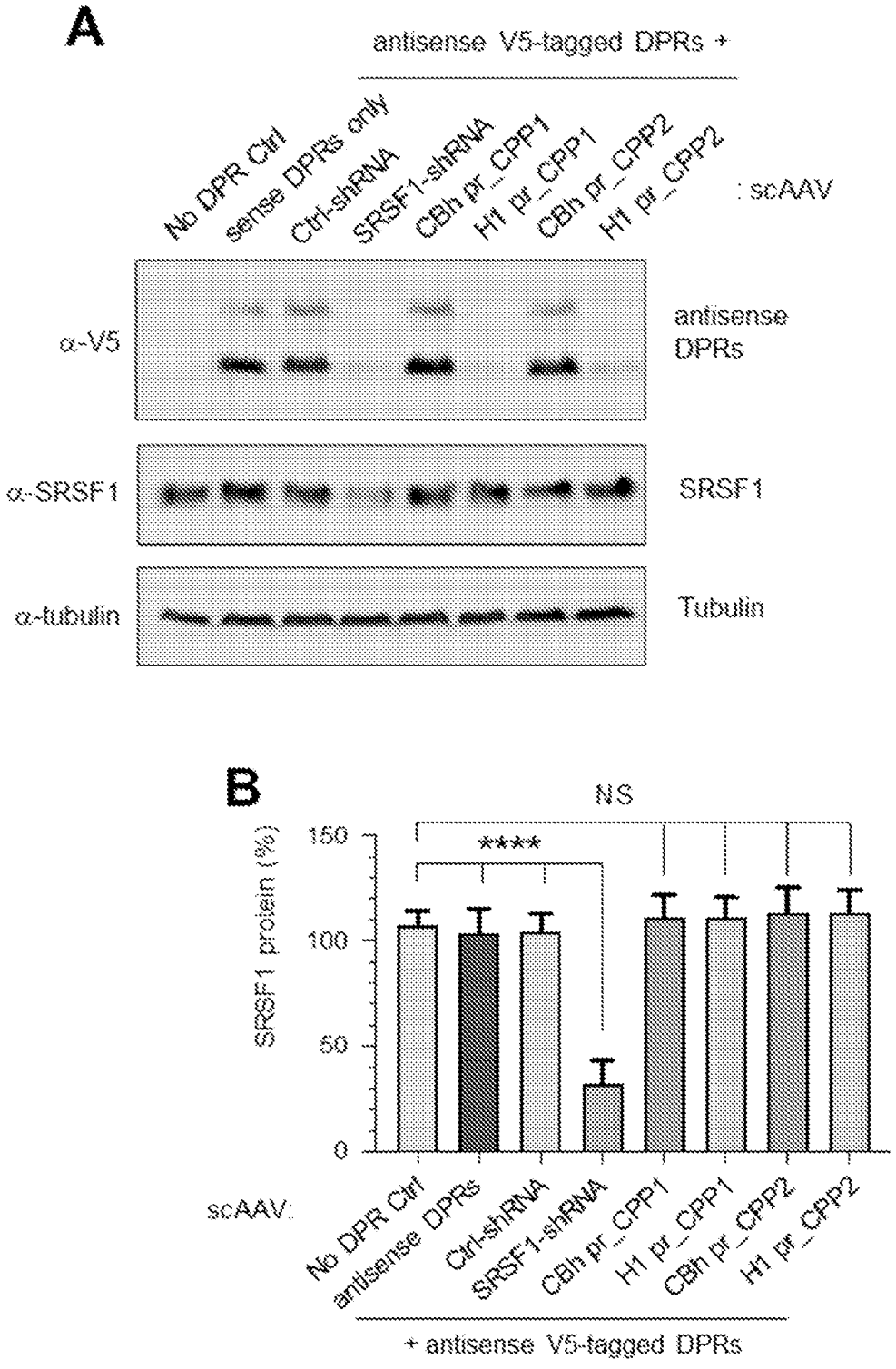

A
antisense V5-tagged DPRs +
No DPR Ctrl
sense DPRs only
Ctrl-shRNA
SRSF1-shRNA
CBh pr_CPP1
H1 pr_CPP1
CBh pr_CPP2
H1 pr_CPP2
: scAAV
α-V5
antisense DPRs
α-SRSF1
SRSF1
α-tubulin
Tubulin
B
NS
****
SRSF1 protein (%)
150
100
50
0
scAAV:
No DPR Ctrl
antisense DPRs
Ctrl-shRNA
SRSF1-shRNA
CBh pr_CPP1
H1 pr_CPP1
CBh pr_CPP2
H1 pr_CPP2
+ antisense V5-tagged DPRs

Figure 10 continued

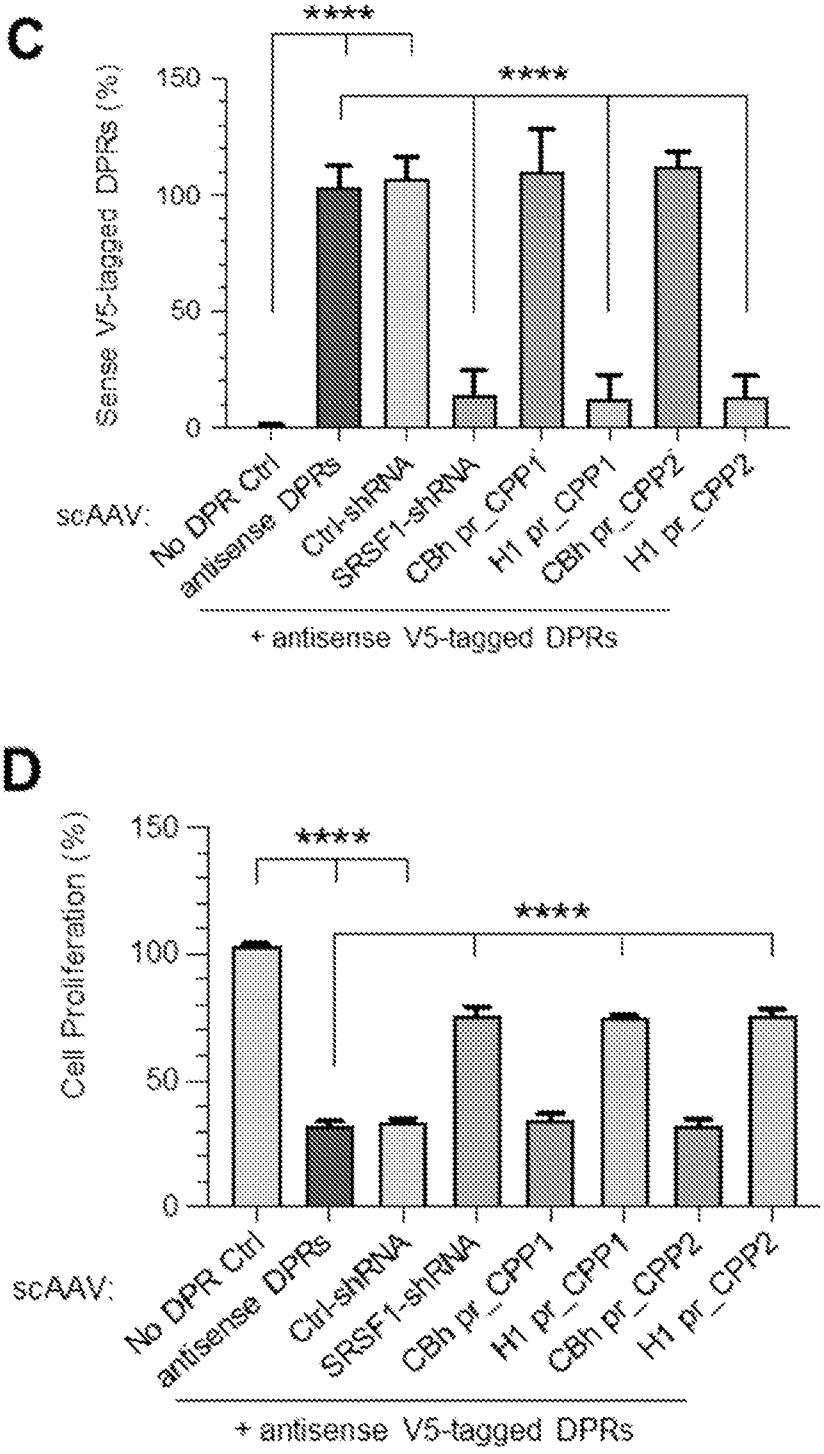
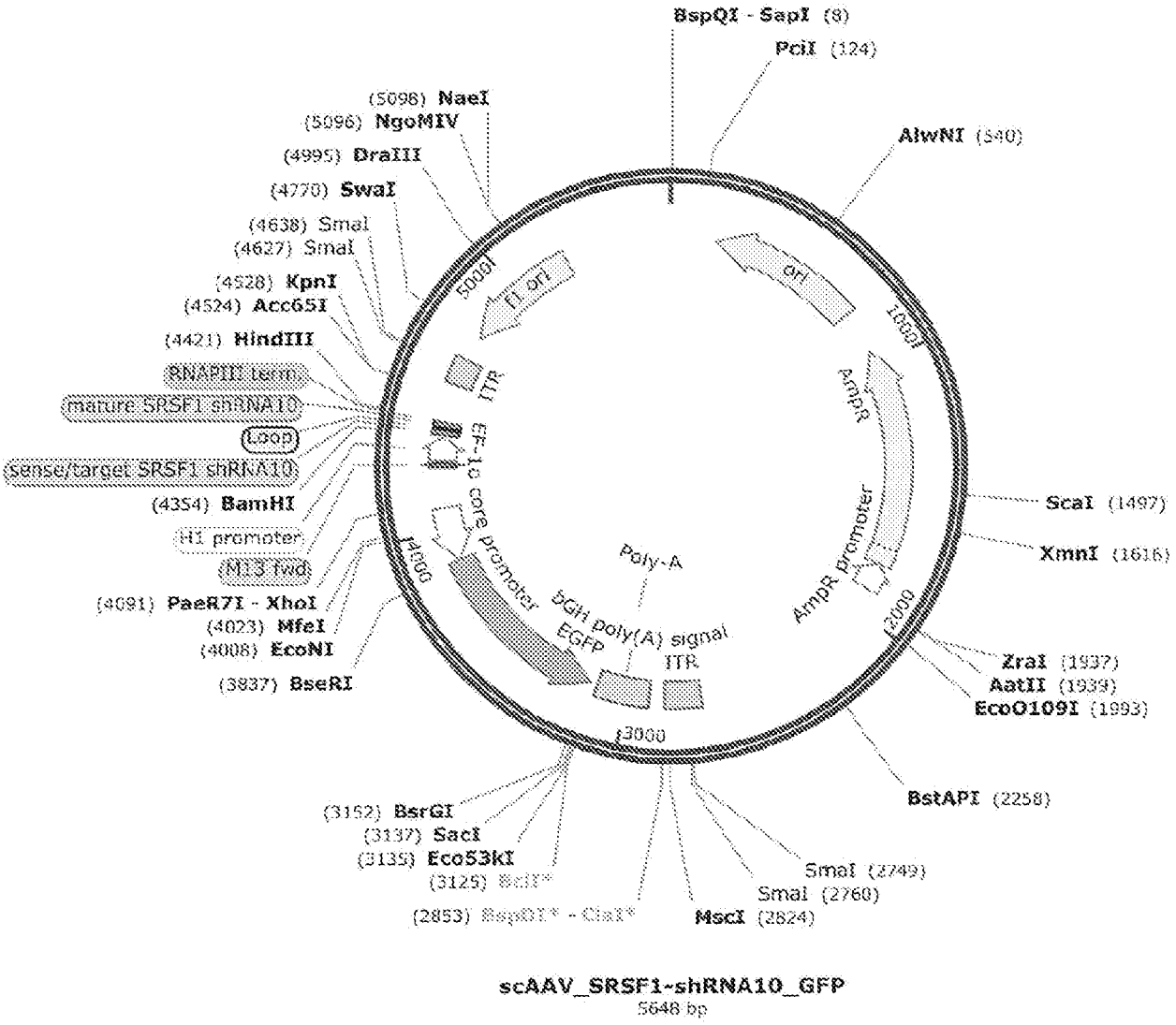


Figure 11


BspQI - SapI (8)
PciI (124)
AlwNI (540)
ori
1000
AmpR
ScaI (1497)
XmnI (1616)
2000
ZraI (1937)
AatII (1939)
EcoO109I (1993)
BstAPI (2258)
SmaI (2749)
SmaI (2760)
MscI (2824)
3000
(3152) BsrGI
(3137) SacI
(3135) Eco53kI
(3129) SciI*
(2853) BspQI* - ClaI*
poly-A
bGH poly(A) signal
ITR
EGFP
EF-1α core promoter
4000
(4091) PaeR7I - XhoI
(4023) MfeI
(4008) EcoNI
(3837) BseRI
(4354) BamHI
H1 promoter
M13 fwd
Sense/target SRSF1 shRNA10
Loop
mature SRSF1 shRNA10
RNAPIII term
(4421) HindIII
(4524) Acc65I
(4528) KpnI
(4627) SmaI
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5648 bp

Figure 11 continued

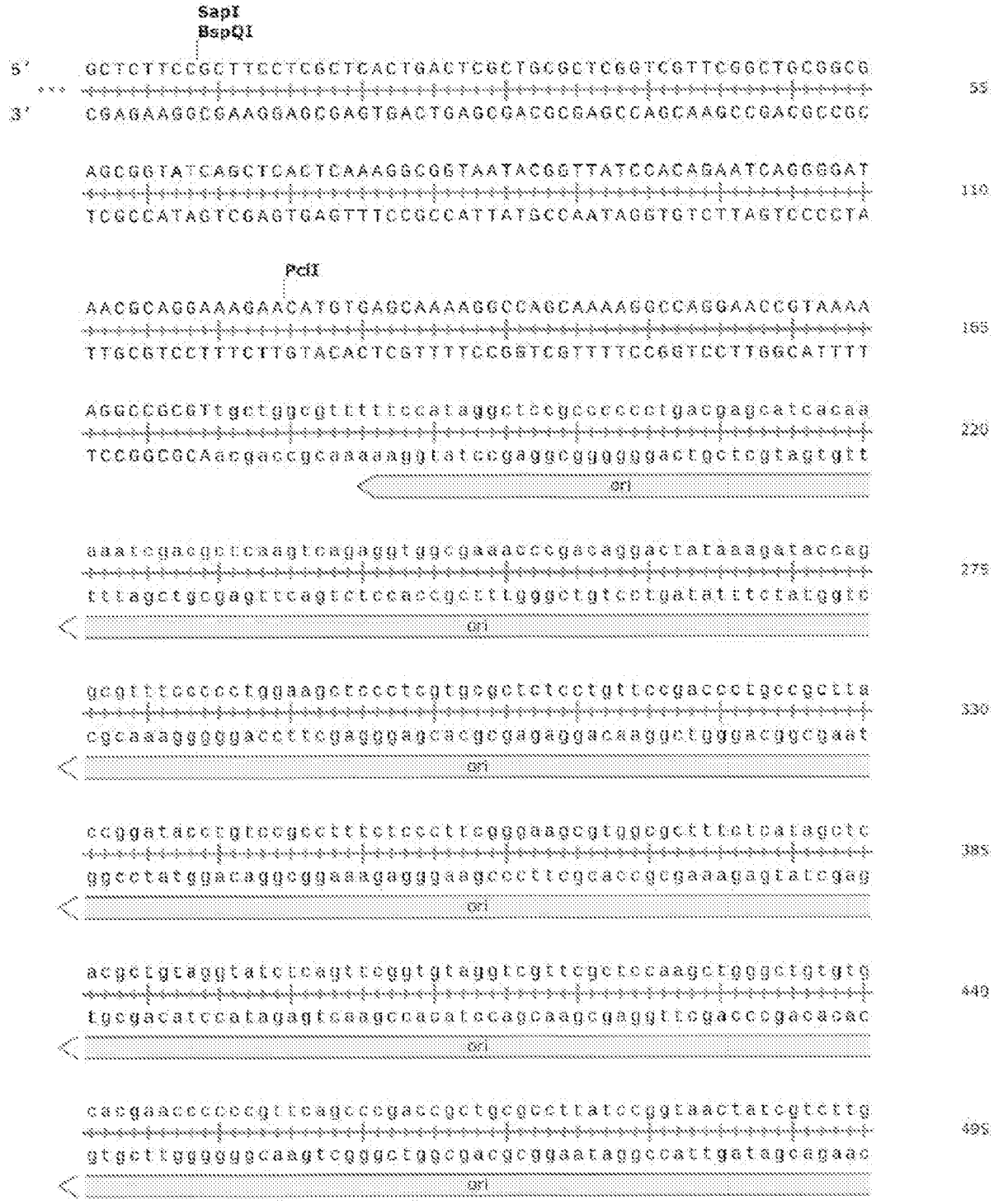


Figure 11 continued

Figure 11 continued

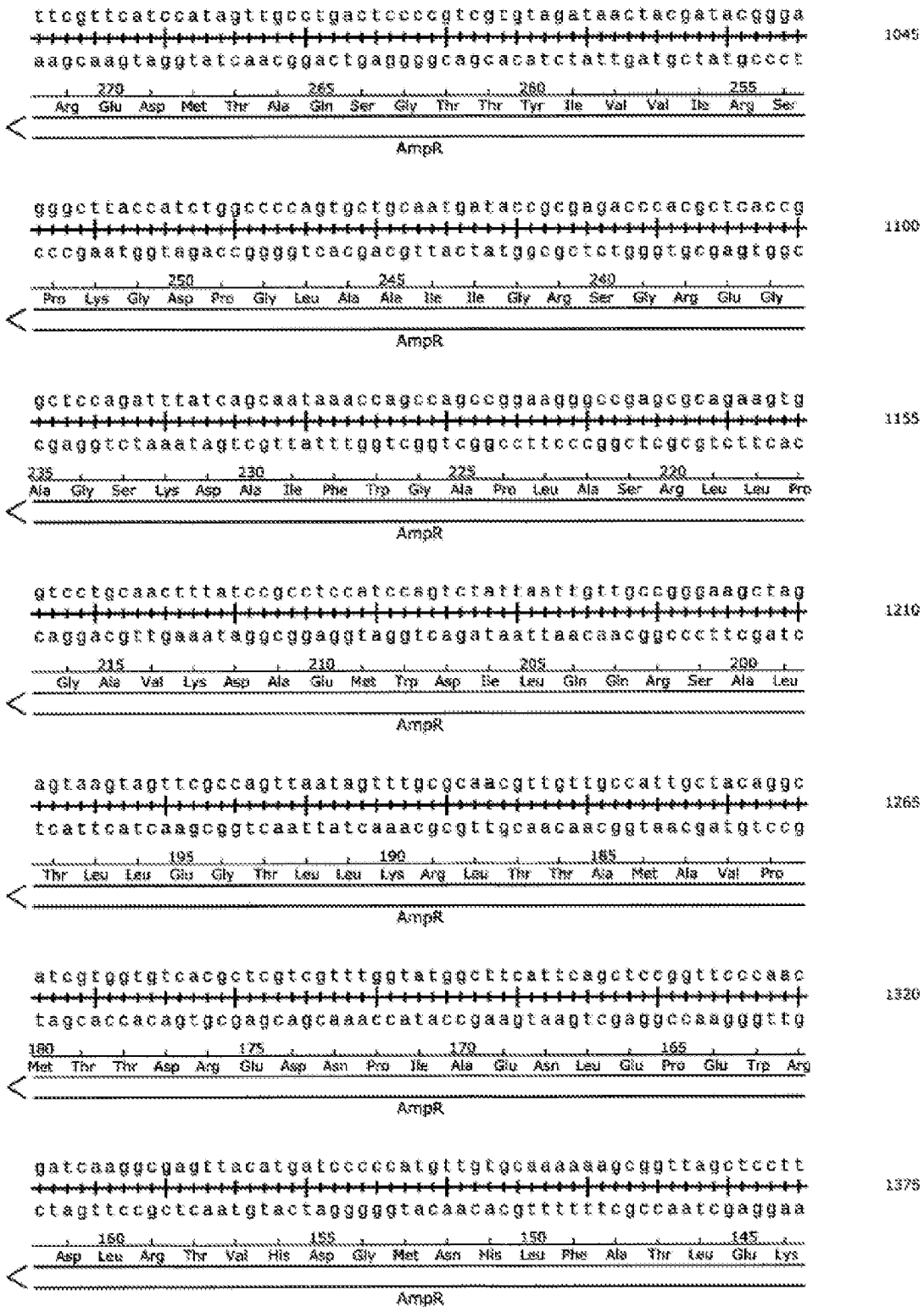
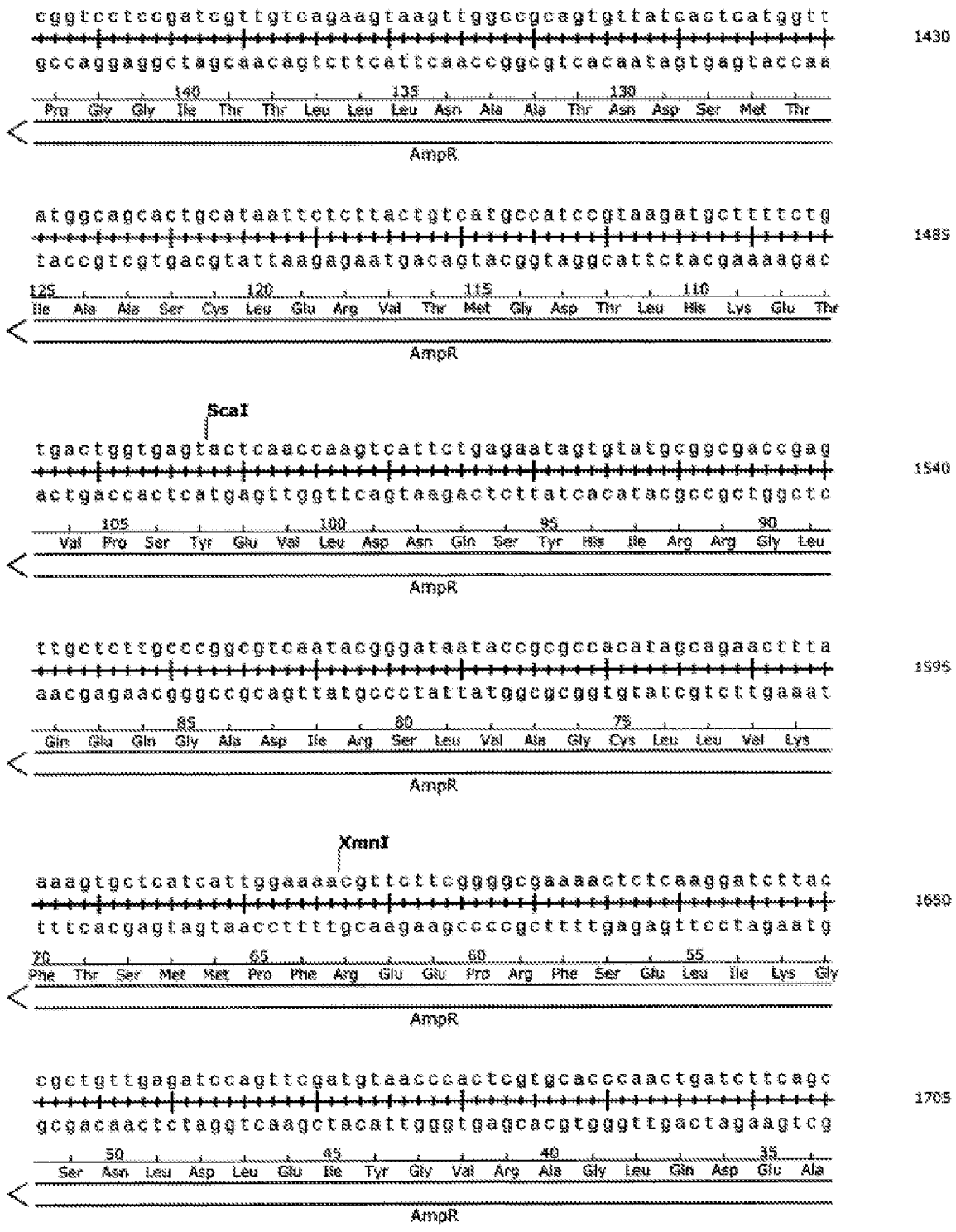


Figure 11 continued



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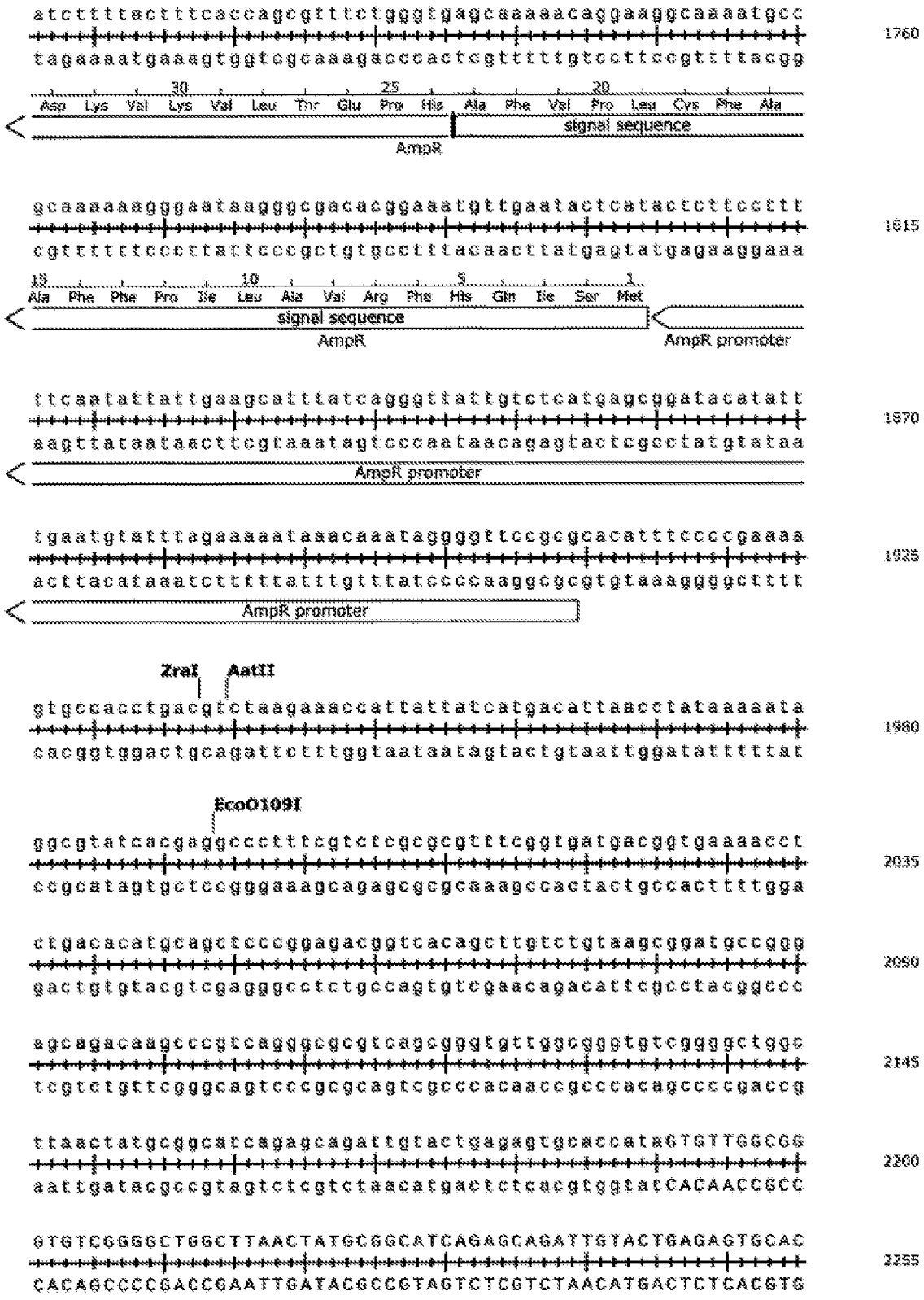

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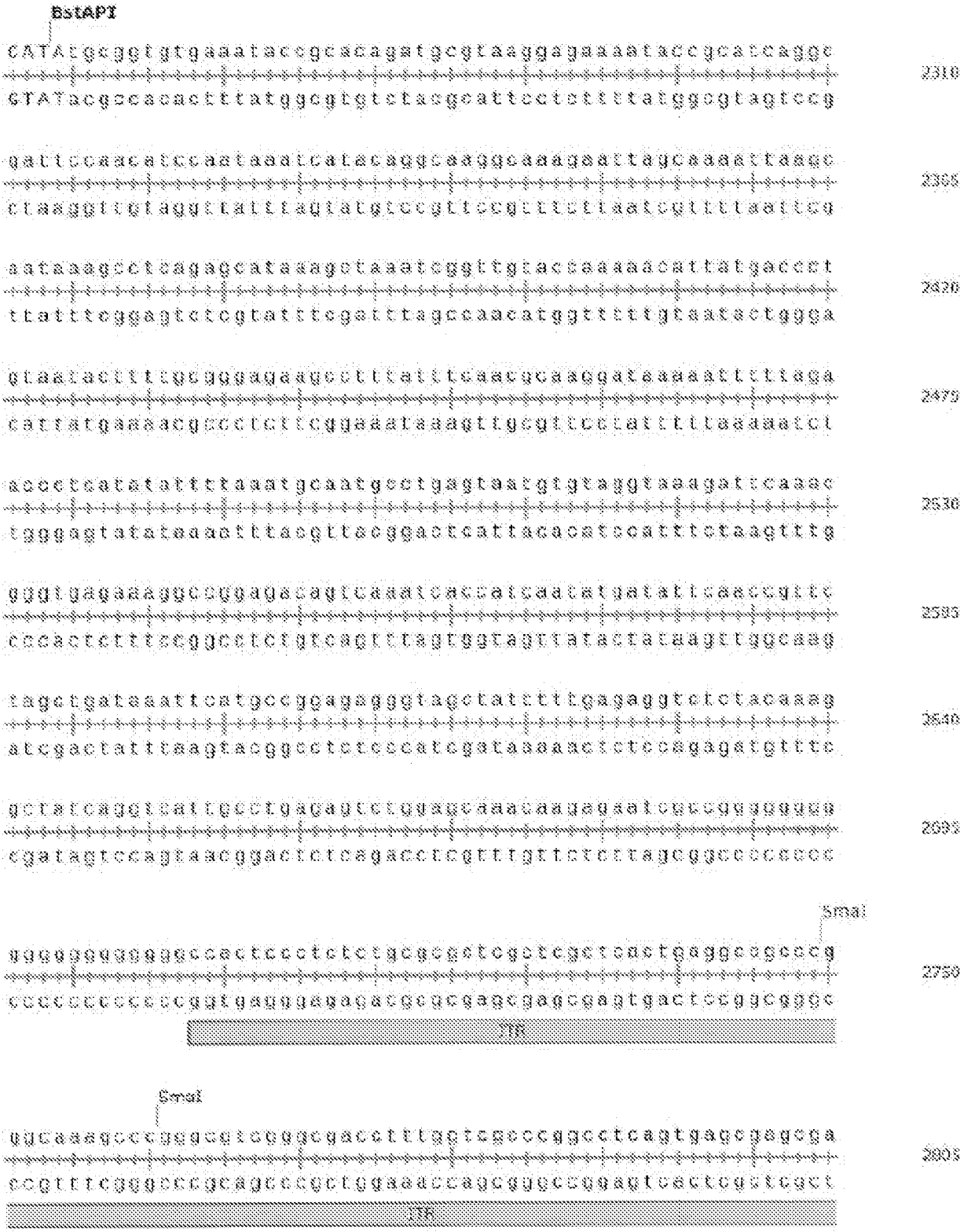

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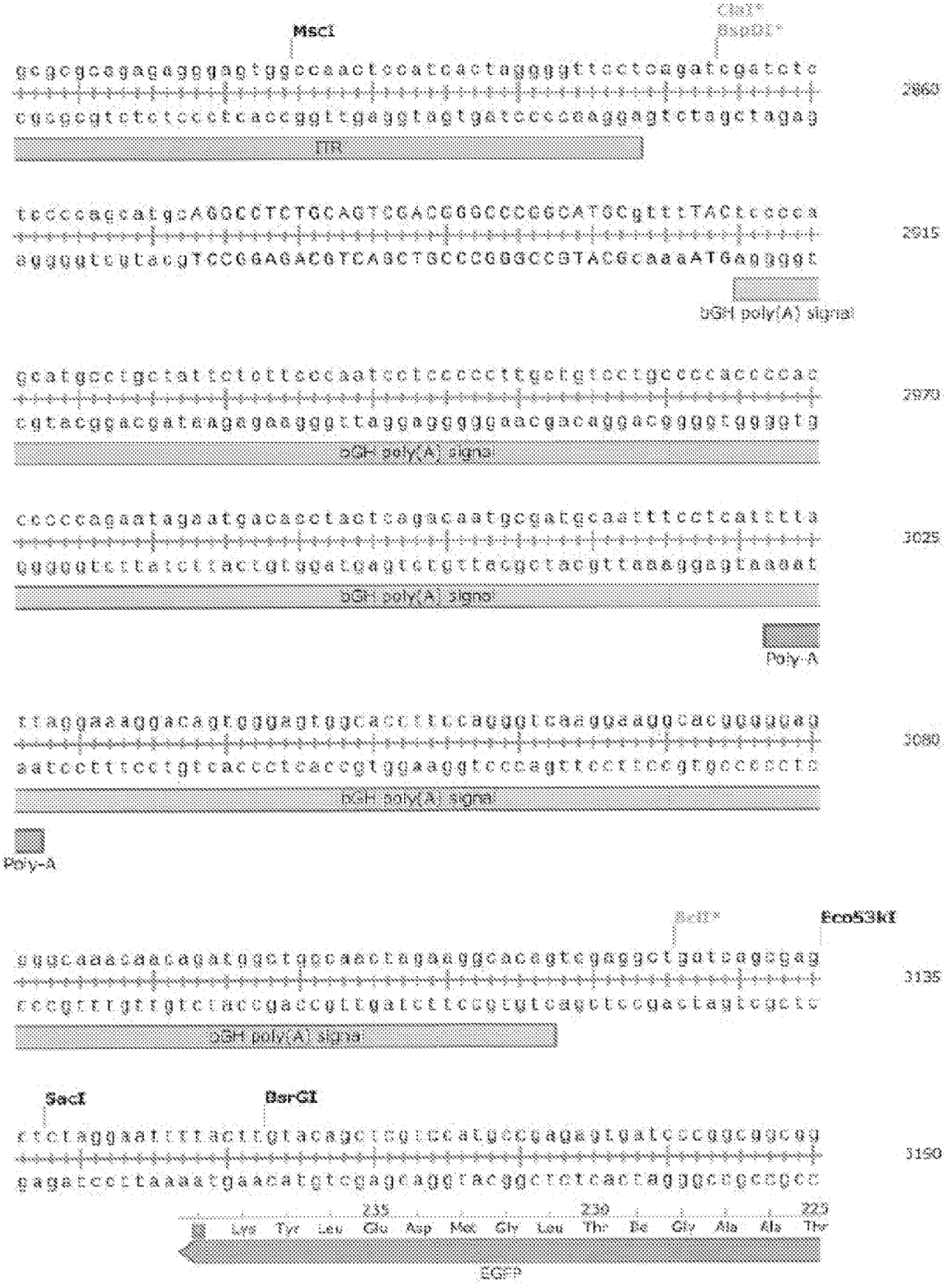

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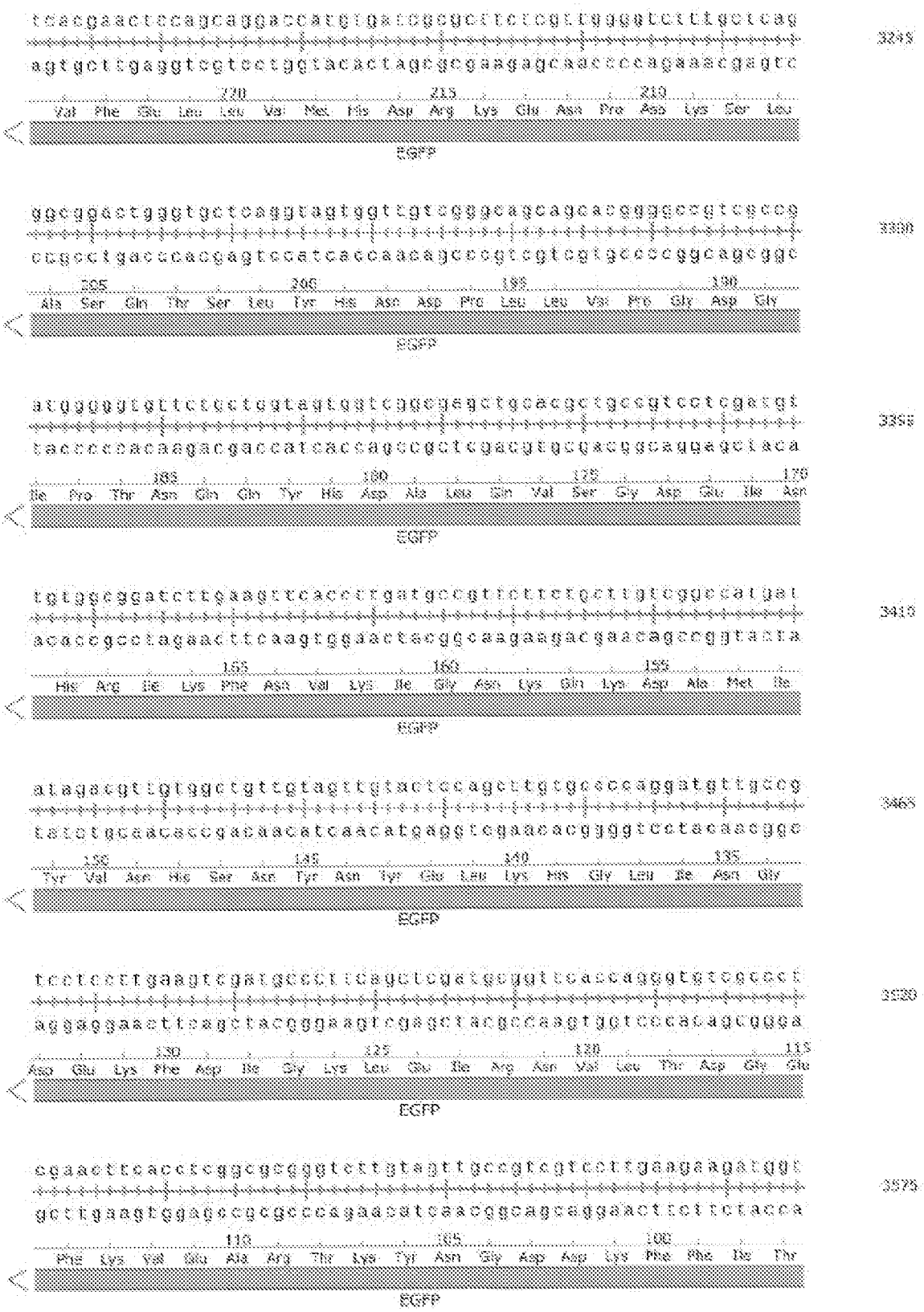

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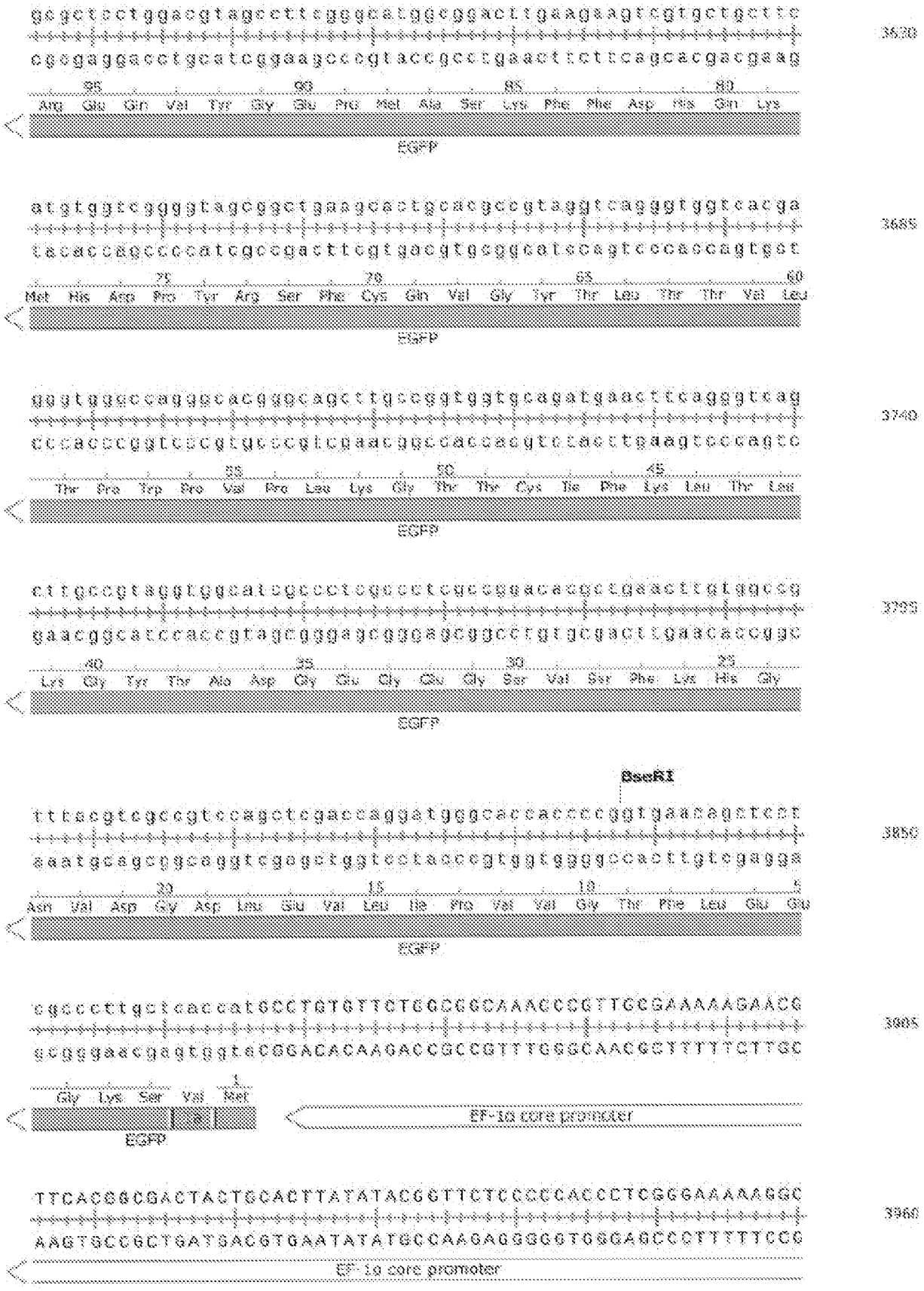

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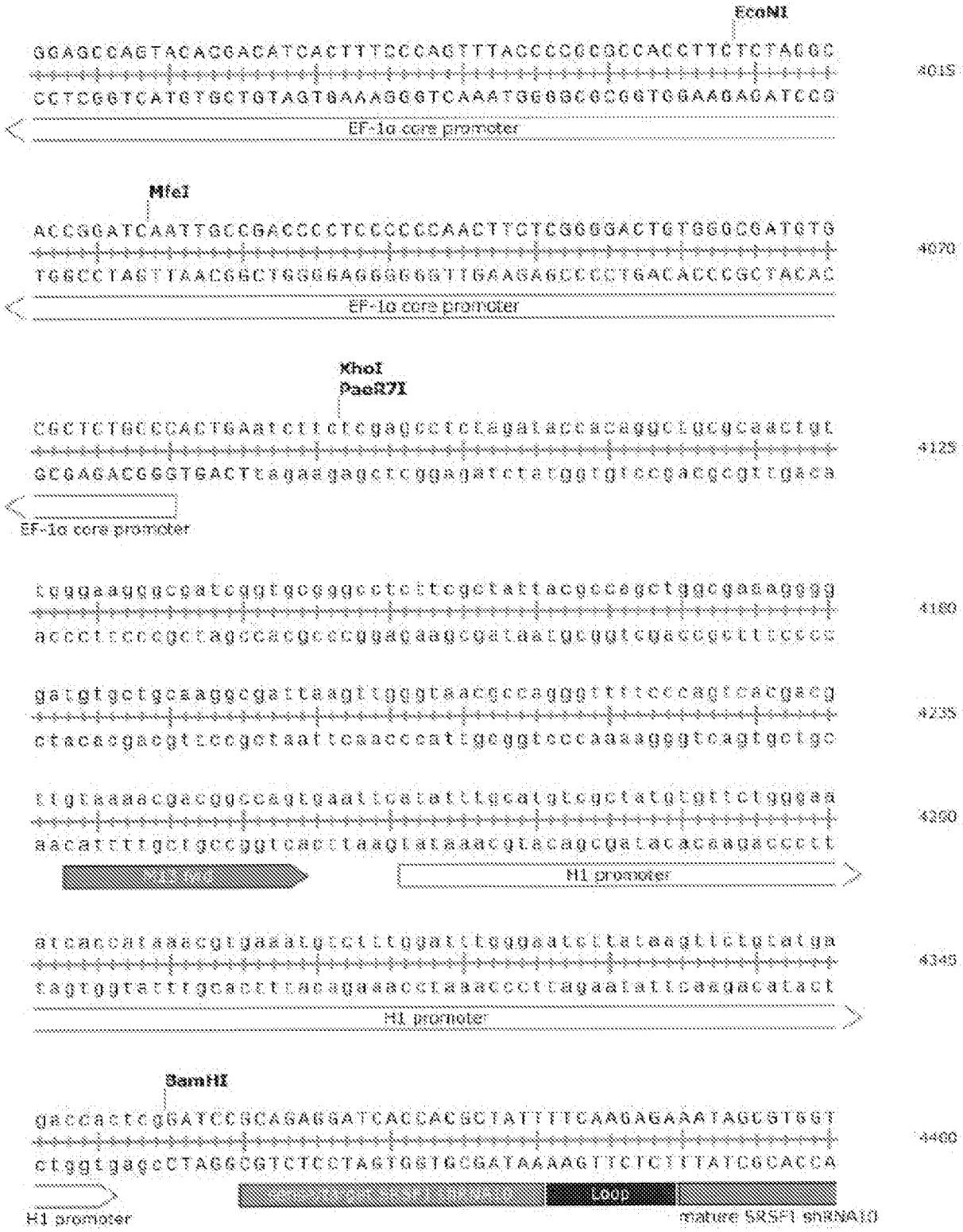
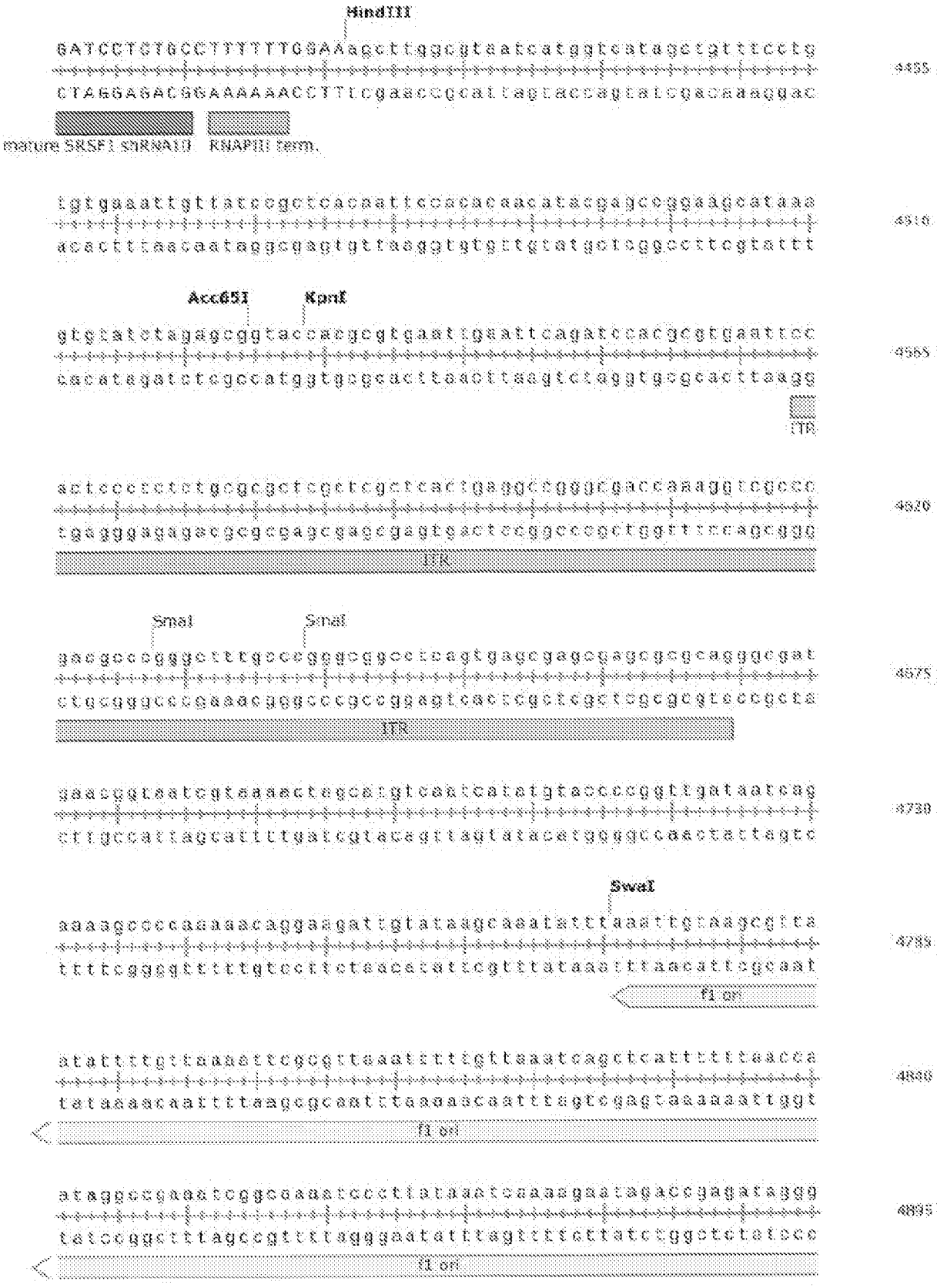


Figure 11 continued



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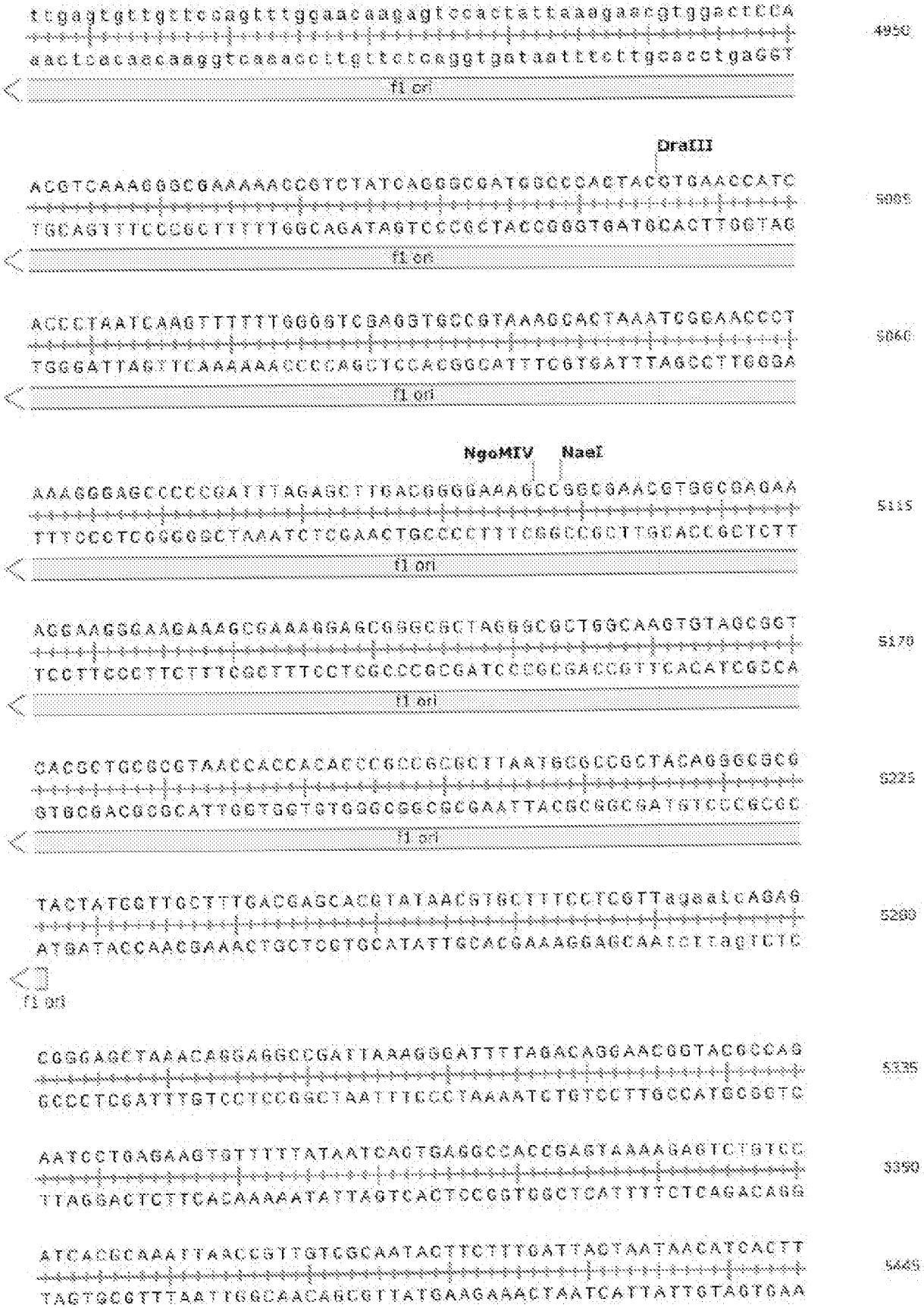

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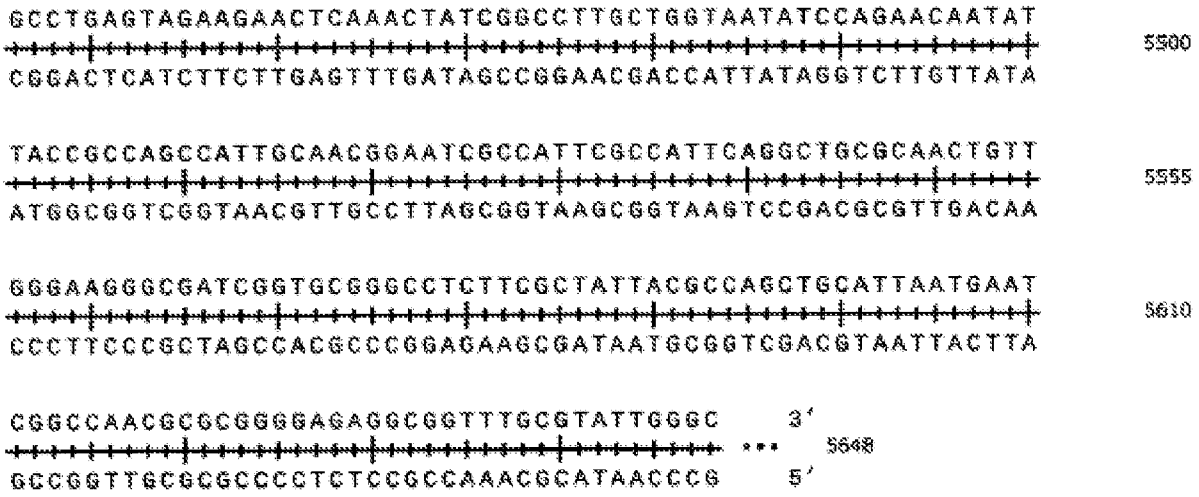


Figure 12

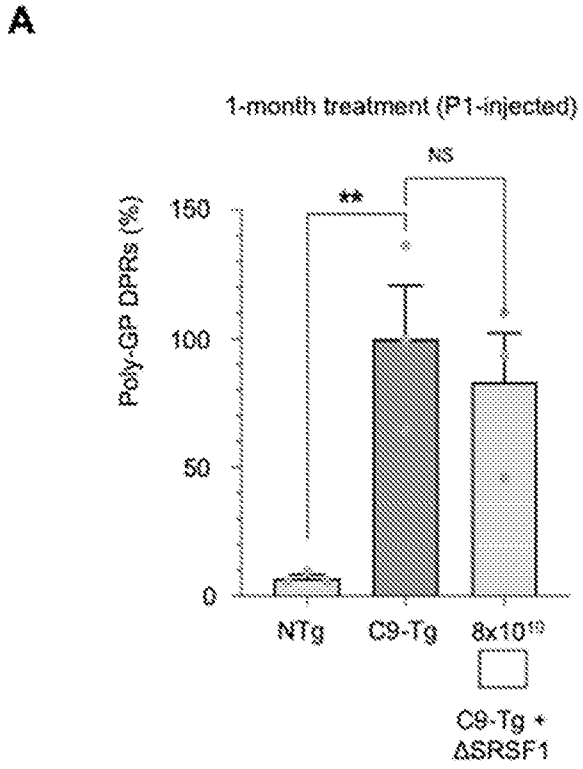


Figure 12 continued

B

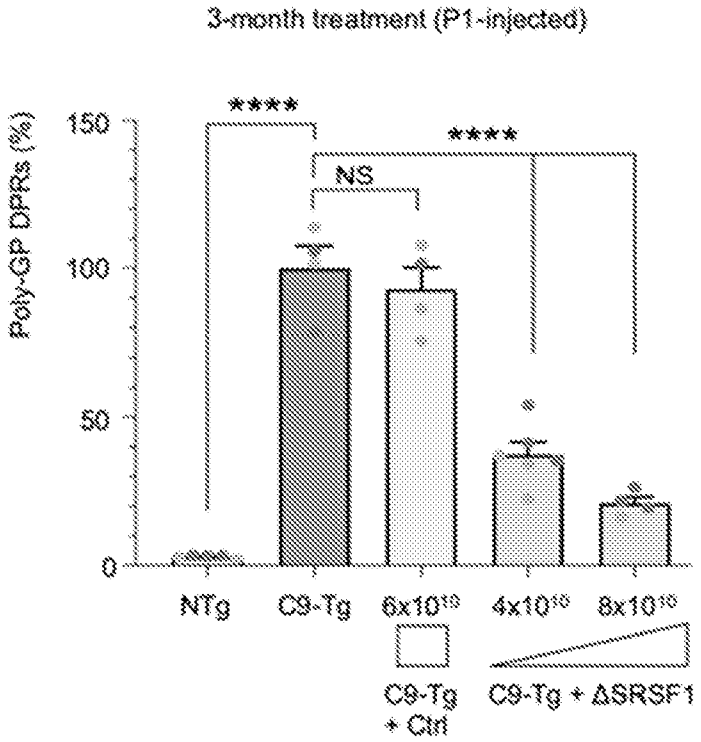


Figure 13

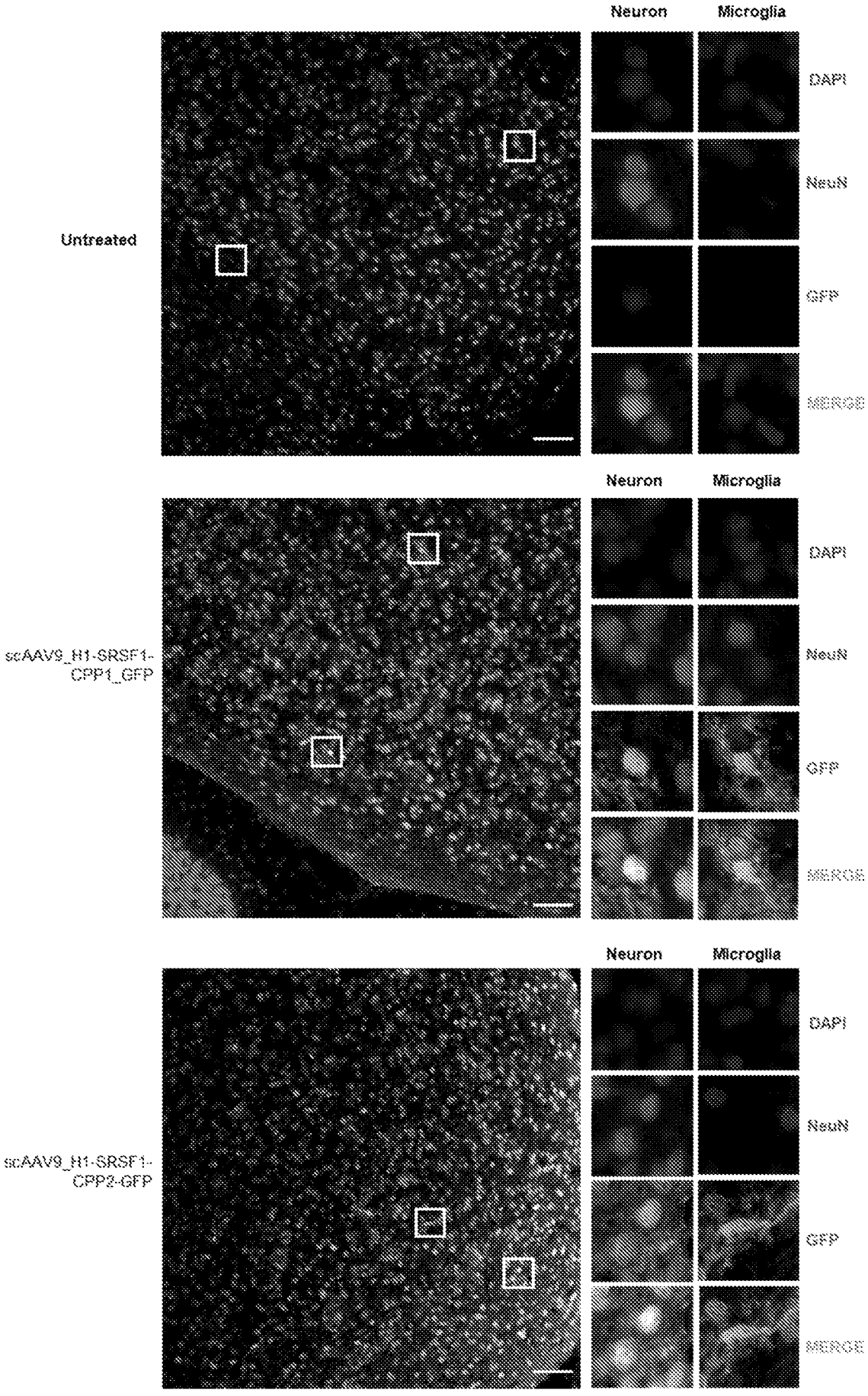
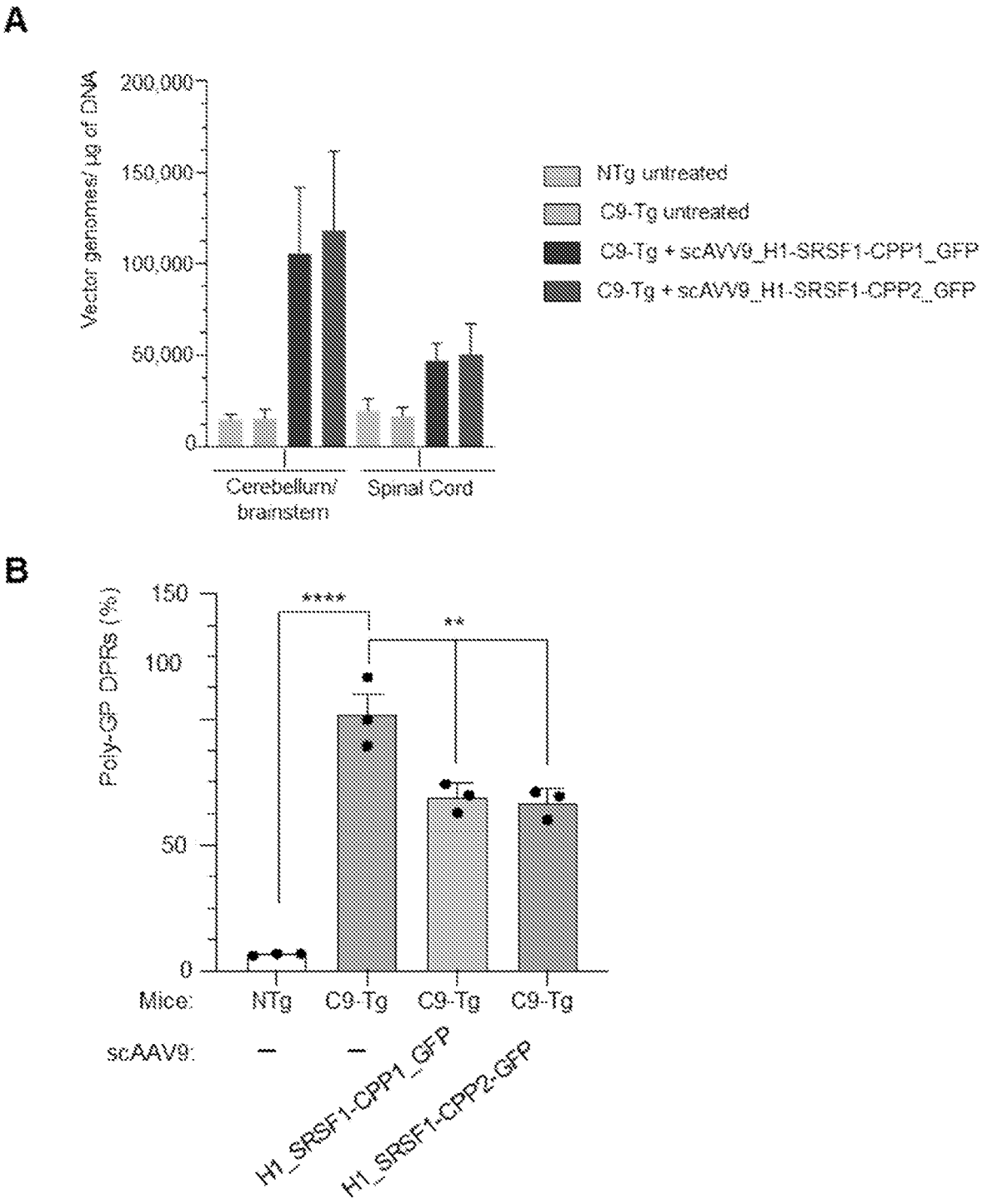

Neuron
Microglia
DAPI
NeuN
GFP
MERGE
Untreated
scAAV9_H1-SRSF1-CPP1_GFP
scAAV9_H1-SRSF1-CPP2-GFP

Figure 14


A
Vector genomes/ µg of DNA
200,000
150,000
100,000
50,000
0
Cerebellum/ brainstem
Spinal Cord
NTg untreated
C9-Tg untreated
C9-Tg + scAVV9_H1-SRSF1-CPP1_GFP
C9-Tg + scAVV9_H1-SRSF1-CPP2_GFP
B
Poly-GP DPRs (%)
150
100
50
0
****
**
Mice:
NTg
C9-Tg
C9-Tg
C9-Tg
scAAV9:
–
–
H1_SRSF1-CPP1_GFP
H1_SRSF1-CPP2-GFP

The following terms are registered trade marks and should be read as such wherever they occur in this document:

Glutamax
Opera
Phenix

Viral Vector

Field of the Disclosure

The present disclosure relates to antagonists that target, directly or indirectly, Serine/Arginine Rich Splicing Factor 1 (SRSF1); viral vectors comprising a nucleic acid sequence encoding SRSF1 antagonists. The use of said vector in gene therapy for the treatment of neurodegenerative diseases such as for example Amyotrophic Lateral Sclerosis (ALS) or sporadic Amyotrophic Lateral Sclerosis which is not caused by a pathological C9ORF72 hexanucleotide repeat expansion and methods thereof are also disclosed.

Background the Disclosure

Gene therapy aims to treat diseases long-term by the introduction of genetic material which alters cell function. Several gene therapy approaches exist such as the delivery of a functional gene to replace a faulty one, inactivation of toxic genes through gene silencing or antisense, introduction or overexpression of genes absent in the host and gene editing approaches. The genetic material is most commonly delivered using viral based vectors such as adenoviruses (Ads), adeno-associated virus (AAVs), self-complementary AAVs and retroviruses i.e. lentiviruses.

The safety of gene therapy vectors requires particular attention as gene therapy vectors persist in the patient's body over a long time and gene therapy vectors must be designed to reduce genotoxic effects, immune reactions or prevent activation of adjacent genes close to the integration site. The backbone of viral vectors typically comprises the protein capsid for packaging the expressed nucleic acid, the genetic information describing the expressed nucleic acid placed between inverted terminal repeats and elements such as promoter elements which allow efficient expression in the host. When delivering genetic material of small size such as short hairpin RNA (shRNA) or antisense oligonucleotides, non-expressed "stuffer" nucleotide sequences are often required to increase the efficiency of shRNA or oligonucleotide nucleic acid targeting, expression and reach optimal packaging capacity.

Neurodegenerative diseases are typically caused by neuronal dysfunction or neuronal loss and affects millions of people worldwide. Neurodegenerative diseases are more prevalent in the aging populations and include but are not limited to amyotrophic lateral sclerosis (ALS), multiple sclerosis, Parkinson's disease, Alzheimer disease, motor neuron and Huntington's disease. ALS and frontotemporal dementia (FTD) are adult-onset neurodegenerative diseases with no effective treatment. ALS is the most common form motor neuron disease (MND), a collective term for a group of neurological disorders characterised by degeneration and loss

of motor neurons. ALS is characterised by selective degeneration of the upper and lower motor neurons, leading to muscle wasting and premature death usually due to respiratory failure and paralysis. Around 90% of ALS cases are classified as sporadic, with approximately 10% showing a genetic component and familial inheritance. FTD is the second most-common form of early-onset dementia characterised by a progressive loss of neuronal cells in frontal and temporal lobe leading to alterations in cognitive function and personality.

The most common genetic cause of ALS and FTD is a hexanucleotide repeat expansion of GGGGCC in the first intron of the chromosome 9 open reading frame 72 (C9orf72) gene, termed C9ALS/FTD.

Antisense oligonucleotide therapies targeting C9ORF72 are in clinical trials and are aimed at reducing the expression of the repeat expansion, thus reducing RNA and DPR toxicity, without affecting the normal expression of C9orf72. Patent US10,801,027 demonstrates that depletion of the export adaptor serine/arginine-rich splicing factor 1 (SRSF1) inhibits the nuclear export of pathological C9ORF72 repeat transcripts retaining hexanucleotide repeat expansions and is hereby incorporated by reference.

However, although depletion of SRSF1 works in patients with ALS caused by hexanucleotide repeat expansions, the present disclosure identified that depletion of SRSF1 also confers neuroprotection in sporadic ALS cases which are not caused by a pathological C9ORF72 hexanucleotide repeat expansion.

Statement of the Invention

According to an aspect of the invention there is provided a viral vector comprising a transcription cassette for the expression of a nucleic acid molecule in a mammalian host cell wherein said nucleic acid molecule is operably linked to a promoter adapted to express said nucleic acid molecule in said mammalian host cell characterised in that said vector comprises a non-expressed nucleotide sequence and wherein said nucleic acid molecule encodes an antagonistic agent that targets Serin/Arginine Rich Splice Factor (SRSF1) or an SRSF1 peptide sequence.

The non-expressed nucleotide sequence is typically referred to a “Stuffer” sequence. Stuffer nucleotide sequences are known in the art and are non-expressed nucleotide sequences that provide optimal viral packaging of viral based vectors. Stuffer sequences are disclosed in PCT/US2013/031644 and is hereby incorporated by reference in its entirety. Stuffer nucleotide

sequences can be placed between the viral inverted terminal repeat sequences, either side of the transgene of interest or two stuffer sequences could be added on each side of the transgene of interest.

In a preferred embodiment of the invention said antagonistic agent is a polypeptide or peptide.

In a preferred embodiment of the invention said antagonistic agent is a nucleic acid-based agent.

In a preferred embodiment of the invention said nucleic acid-based agent is an antisense nucleic acid, an inhibitory RNA or shRNA or miRNA molecule that is complementary to and inhibits the expression of a nucleic acid encoding a Serin/Arginine Rich Splice Factor (SRSF1).

Preferably said SRSF1 comprises or consist of a sequence set forth in SEQ ID NO 67.

Alternatively, said SRSF1 comprises or consist of a sequence set forth in SEQ ID NO 76.

The nucleic acid-based agent is designed with reference to the sequence set forth in SEQ ID NO 67, or alternatively with reference to the sequence set forth in SEQ ID NO 76.

In a preferred embodiment of the invention said nucleic acid-based agent is an inhibitory RNA.

In a preferred embodiment of the invention said nucleic acid-based agent is an antisense RNA.

In a further preferred embodiment of the invention said inhibitory RNA is a shRNA or miRNA molecule.

A technique to specifically ablate gene function is through the introduction of double stranded RNA, also referred to as small inhibitory or interfering RNA (siRNA, shRNA and miRNA), into a cell which results in the destruction of mRNA complementary to the sequence included in the siRNA molecule. The siRNA molecule comprises two complementary strands of RNA (a sense strand and an antisense strand) annealed to each other to form a double stranded RNA molecule. The siRNA molecule is typically derived from exons of the gene which is to be ablated. The mechanism of RNA interference is being elucidated. Many organisms respond to the presence of double stranded RNA by activating a cascade that leads to the formation of siRNA. The presence of double stranded RNA activates a protein complex comprising RNase III which processes the double stranded RNA into smaller fragments (siRNAs,

approximately 21-29 nucleotides in length) which become part of a ribonucleoprotein complex. The siRNA acts as a guide for the RNase complex to cleave mRNA complementary to the antisense strand of the siRNA thereby resulting in destruction of the mRNA.

In a preferred embodiment of the invention said inhibitory RNA molecule is between 19 nucleotides [nt] and 29nt in length. More preferably still said inhibitory RNA molecule is between 21nt and 27nt in length. Preferably said inhibitory RNA molecule is about 21nt in length.

In a preferred embodiment of the invention said inhibitory RNA comprises or consists of a nucleotide sequence as set forth in SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 or 58.

In a preferred embodiment of the invention said shRNA comprises or consist of a nucleotide sequence selected from the group consisting of SEQ ID NO 2, 3, 4, 5, 6, 7, 8, 9,10 and 11.

In a preferred embodiment of the invention said shRNA comprises or consist of a nucleotide sequence set forth in SEQ ID NO 7.

In a preferred embodiment of the invention said shRNA comprises or consist of a nucleotide sequence set forth in SEQ ID NO 10.

In a preferred embodiment of the invention said shRNA comprises or consist of a nucleotide sequence set forth in SEQ ID NO 11.

In a preferred embodiment of the invention said peptide comprises an amino acid sequence that is at least 10 amino acids in length and comprises all or part of the amino acid sequence set forth in SEQ ID NO: 59.

In a preferred embodiment of the invention said peptide comprises an amino acid sequence that is at least 32 amino acids in length and comprises the amino acid sequence set forth in SEQ ID NO: 59.

In a preferred embodiment of the invention said peptide is at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 29, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or at least 100 amino

acids in length but less than the full-length amino acid sequence set forth in SEQ ID NO: 60 or 61.

In a preferred embodiment of the invention said peptide consists of an amino sequence as set forth in SEQ ID NO: 59.

In an alternative embodiment of the invention said peptide is a dominant negative protein comprising a modification of the amino acid sequence set forth in SEQ ID NO: 60 or 61.

In a preferred embodiment of the invention said dominant negative protein comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 60 or 61 wherein said amino acid sequence is modified by addition, deletion or substitution of one or more amino acid residues.

In a preferred embodiment of the invention said modified protein comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 62 or 63.

In a preferred embodiment of the invention said nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide or peptide is set forth set forth in SEQ ID NO: 89, or a sequence which is to 90% identical to the sequence set forth in SEQ ID NO 89.

In a further preferred embodiment of the invention said nucleic acid sequence is at least 36 nucleic acids in length.

In a preferred embodiment of the invention said peptide comprises an amino acid sequence that is at least 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40 or 42 amino acids in length and set forth in SEQ ID NO: 90.

In a preferred embodiment of the invention said peptide comprises an amino acid sequence that is set forth in SEQ ID NO: 75 (GSWQDLKDHMREA).

In a preferred embodiment of the invention said viral vector comprises a RNA Pol III terminator.

Preferably said terminator comprises the nucleic acid sequence 5' TTTTTT 3'.

In a preferred embodiment of the invention said vector comprises inverted terminal repeat nucleotide sequences.

Inverted terminal repeat sequences (ITR) are typically positioned upstream and downstream of a transcription cassette. Alternatively, the ITRs are upstream and downstream of the transcription cassette, the non-expressed nucleotide sequence and any optional regulatory elements.

In a preferred embodiment of the invention said ITR sequence is set forth in SEQ ID NO 64.

In a preferred embodiment of the invention said ITR sequence is set forth in SEQ ID NO 88.

In a preferred embodiment of the invention said promoter is selected from the group consisting of H1 Polymerase III promoter, U6 promoter, U7 promoter or the mammalian 7SK promoter.

In a further preferred embodiment of the invention said promoter is a H1 Polymerase III promoter.

In a preferred embodiment said H1 Polymerase III promoter is set forth in SEQ ID NO 65.

Viruses are commonly used as vectors for the delivery of exogenous genes. Commonly employed vectors include recombinantly modified enveloped or non-enveloped DNA and RNA viruses, for example baculoviridiae, parvoviridiae, picornoviridiae, herpesveridiae, poxviridae, adenoviridiae, picornnaviridiae or retroviridae e.g. lentivirus. Chimeric vectors may also be employed which exploit advantageous elements of each of the parent vector properties (See e.g., Feng, et al (1997) Nature Biotechnology 15:866-870). Such viral vectors may be wild-type or may be modified by recombinant DNA techniques to be replication deficient, conditionally replicating or replication competent. Conditionally replicating viral vectors are used to achieve selective expression in particular cell types while avoiding untoward broad-spectrum infection. Examples of conditionally replicating vectors are described in Pennisi, E. (1996) Science 274:342-343; Russell, and S.J. (1994) Eur. J. of Cancer 30A(8):1165-1171.

Preferred viral vectors are derived from the adenoviral, adeno-associated viral or retroviral genomes.

In a preferred embodiment of the invention said viral based vector is an adeno-associated virus [AAV].

In a preferred embodiment of the invention said adeno-associated virus is a self-complementary adeno-associated virus (scAAV).

In a preferred embodiment said viral based vector is selected from the group consisting of: AAV2, AAV3, AAV6, AAV13; AAV1, AAV4, AAV5, AAV6, AAV9 and AAVrh10.

In a preferred embodiment said scAAV is selected from the group consisting of: scAAV2, scAAV3, scAAV6, scAAV13; scAAV1, scAAV4, scAAV5, scAAV6, scAAV9 and scAAVrh10.

In a preferred embodiment of the invention said viral based vector is scAAV9 or scAAVrh10.

In an alternative preferred embodiment of the invention said viral based vector is a lentiviral vector.

According to a further aspect of the invention there is provided a pharmaceutical composition comprising a viral vector according to the invention and an excipient or carrier.

The viral vector compositions of the present invention are administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers and supplementary therapeutic agents. The expression vector compositions of the invention can be administered by any conventional route, including injection or by gradual infusion over time and in particular intrathecal (e.g., lumbar puncture) and/or intracerebral.

The viral vector compositions of the invention are administered in effective amounts. An "effective amount" is that amount of the expression vector that alone, or together with further doses, produces the desired response. In the case of treating a disease, the desired response is inhibiting the progression of the disease. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine methods. Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical

judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

The viral vector compositions used in the foregoing methods preferably are sterile and contain an effective amount of expression vector according to the invention for producing the desired response in a unit of weight or volume suitable for administration to a patient. The doses of vector administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of administration used and the state of the subject. Other factors include the desired period of treatment. If a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Other protocols for the administration of vector compositions will be known to one of ordinary skill in the art, in which the dose amount, schedule of injections, sites of injections, mode of administration and the like vary from the foregoing. The administration of compositions to mammals other than humans, (e.g. for testing purposes or veterinary therapeutic purposes), is carried out under substantially the same conditions as described above. A subject, as used herein, is a mammal, preferably a human, and including a non-human primate, cow, horse, pig, sheep, goat, dog, cat or rodent.

When administered, the viral vector compositions of the invention are applied in pharmaceutically acceptable amounts and in pharmaceutically acceptable compositions. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active agent. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents' (e.g. those typically used in the treatment of the specific disease indication). When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

The pharmaceutical compositions containing the viral vectors according to the invention may contain suitable buffering agents, including acetic acid in a salt; citric acid in a salt; boric acid

in a salt; and phosphoric acid in a salt. The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

The viral vector compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a vector which constitutes one or more accessory ingredients. The preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1, 3-butanediol. Among the acceptable solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

According to a further aspect of the invention there is provided a viral vector according to the invention for use as a medicament.

According to a further aspect of the invention there is provided a viral vector according to the invention for use in the treatment of a neurodegenerative disease.

In a preferred embodiment of the invention said neurodegenerative disease is selected from the group consisting of: amyotrophic lateral sclerosis (ALS) sporadic amyotrophic lateral sclerosis, familial ALS caused by a mutation other than a pathological *C9ORF72*-repeat expansion, frontotemporal dementia (FTD) motor neurone disease, frontotemporal lobar dementia (FTLD), Huntington's like disorder, and Fragile X-associated tremor/ataxia syndrome (FXTAS).

In a preferred embodiment of the invention said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

In a preferred embodiment of the invention said neurodegenerative disease is sporadic and/or familial amyotrophic lateral sclerosis.

In a preferred embodiment of the invention said neurodegenerative disease is ALS not caused by pathological C9ORF72-repeat expansions

In a preferred embodiment of the invention said neurodegenerative disease is sporadic frontotemporal dementia (FTD).

In a preferred embodiment of the invention said neurodegenerative disease is Fragile X-associated tremor/ataxia syndrome (FXTAS).

According to a further aspect of the invention there is provided a cell transfected with a viral vector according to the invention.

In a preferred embodiment of the invention said cell is a neurone and/or an astrocyte.

In a preferred embodiment of the invention said neurone is a motor neurone and/or an astrocyte.

According to a further aspect of the invention there is provided a method to treat or prevent a neurodegenerative disease comprising administering a therapeutically effective amount of a viral vector according to the invention to prevent and/or treat said neurodegenerative disease.

In a preferred method of the invention said neurodegenerative disease is sporadic amyotrophic lateral sclerosis and familial amyotrophic lateral sclerosis.

In a preferred method of the invention said neurodegenerative disease is amyotrophic lateral sclerosis.

In a preferred embodiment of the invention said neurodegenerative disease is ALS not caused by pathological C9ORF72-repeat expansions.

In a preferred method of the invention said neurodegenerative disease is sporadic frontotemporal dementia (FTD).

In a preferred method of the invention said neurodegenerative disease is Fragile X-associated tremor/ataxia syndrome (FXTAS).

According to a further aspect of the invention there is provided an isolated nucleic acid molecule encoding an shRNA molecule comprising or consisting of a nucleotide sequence selected from the group consisting of SEQ ID NO 2, 3, 4, 5, 6, 7, 8, 9,10 and 11.

The invention includes sequence variants corresponding to the recited SEQ ID. A sequence variant is one that varies from a reference sequence by 1, 2, 3, 4 or 5 nucleotide base changes.

In a preferred embodiment of the invention said nucleic acid molecule comprises or consist of a nucleotide sequence set forth in SEQ ID NO 7.

In a preferred embodiment of the invention said nucleic acid molecule comprises or consist of a nucleotide sequence set forth in SEQ ID NO 10.

In a preferred embodiment of the invention said nucleic acid molecule comprises or consist of a nucleotide sequence set forth in SEQ ID NO 11.

According to a further aspect of the invention there is provided shRNA molecules comprising a nucleotide sequence, or variant thereof, selected from the group consisting of:
SRSF1-shRNA1 (SEQ ID NO 91):
GCUGAUGUUUACCGAGAUGGC UUCAAGAGA GCCAUCUCGGUAAACAUCAGC;
SRSF1-shRNA2 (SEQ ID NO 92):
GGAGUUUGUACGGAAAGAAGA UUCAAGAGA UCUUCUUUCCGUACAAACUCC;
SRSF1-shRNA3 (SEQ ID NO 93):
GGAAAGAAGAUAUGACCUAUG UUCAAGAGA CAUAGGUCAUAUCUUCUUUCC;
SRSF1-shRNA4 (SEQ ID NO 94):
GAAAGAAGAUAUGACCUAUGC UUCAAGAGA GCAUAGGUCAUAUCUUCUUUC;
SRSF1-shRNA5 (SEQ ID NO 95):
GCCUACAUCCGGGUUAAAGUU UUCAAGAGA AACUUUAACCCGGAUGUAGGC;
SRSF1-shRNA6 (SEQ ID NO 96):
GGGCCCAGAAGUCCAAGUUAU UUCAAGAGA AUAACUUGGACUUCUGGGCCC;
SRSF1-shRNA7 (SEQ ID NO 97):
GGCCCAGAAGUCCAAGUUAUG UUCAAGAGA CAUAACUUGGACUUCUGGGCC;
SRSF1-shRNA8 (SEQ ID NO 98):
GCCCAGAAGUCCAAGUUAUGG UUCAAGAGA CCAUAACUUGGACUUCUGGGC;
SRSF1-shRNA9 (SEQ ID NO 99):
GGAAGAUCUCGAUCUCGAAGC UUCAAGAGA GCUUCGAGAUCGAGAUCUUCC; and
SRSF1-shRNA10 (SEQ ID NO 100):

GCAGAGGAUCACCACGCUAUU UUCAAGAGA AAUAGCGUGGUGAUCCUCUGC.

In a preferred embodiment of the invention said shRNA molecule comprises or consists of a nucleotide sequence, or variant thereof, set forth in SEQ ID NO 96.

In a preferred embodiment of the invention said shRNA molecule comprises or consists of a nucleotide sequence, or variant thereof, set forth in SEQ ID NO 99.

In a preferred embodiment of the invention said shRNA molecule comprises or consists of a nucleotide sequence, or variant thereof, set forth in SEQ ID NO 100.

According to an aspect of the invention there is provided an isolated nucleic acid molecule or shRNA according to the invention for use as a medicament.

According to a further aspect of the invention there is provided an isolated nucleic acid molecule or shRNA according to the invention for use in the treatment of a neurodegenerative disease.

In a preferred embodiment of the invention said neurodegenerative disease is selected from the group consisting of: amyotrophic lateral sclerosis (ALS) sporadic amyotrophic lateral sclerosis, familial ALS caused by a mutation other than a pathological C9ORF72-repeat expansion, frontotemporal dementia (FTD) motor neurone disease, frontotemporal lobar dementia (FTLD), Huntington's like disorder, and Fragile X-associated tremor/ataxia syndrome (FXTAS).

In a preferred embodiment of the invention said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

In a preferred embodiment of the invention said neurodegenerative disease is sporadic and/or familial amyotrophic lateral sclerosis.

In a preferred embodiment of the invention said neurodegenerative disease is ALS not caused by pathological C9ORF72-repeat expansions

In a preferred embodiment of the invention said neurodegenerative disease is sporadic frontotemporal dementia (FTD).

In a preferred embodiment of the invention said neurodegenerative disease is Fragile X-associated tremor/ataxia syndrome (FXTAS).

According to an aspect of the invention there is provided an siRNA molecule comprising or consisting of a nucleic acid sequence designed with reference to the shRNA set forth in SEQ ID NO 77-86.

According to an aspect of the invention there is provided an siRNA molecule according to the invention for use as a medicament.

According to a further aspect of the invention there is provided an siRNA molecule according to the invention for use in the treatment of a neurodegenerative disease.

In a preferred embodiment of the invention said neurodegenerative disease is selected from the group consisting of: amyotrophic lateral sclerosis (ALS) sporadic amyotrophic lateral sclerosis, familial ALS caused by a mutation other than a pathological C9ORF72-repeat expansion, frontotemporal dementia (FTD) motor neurone disease, frontotemporal lobar dementia (FTLD), Huntington's like disorder, and Fragile X-associated tremor/ataxia syndrome (FXTAS).

In a preferred embodiment of the invention said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

In a preferred embodiment of the invention said neurodegenerative disease is sporadic and/or familial amyotrophic lateral sclerosis.

In a preferred embodiment of the invention said neurodegenerative disease is ALS not caused by pathological C9ORF72-repeat expansions

In a preferred embodiment of the invention said neurodegenerative disease is sporadic frontotemporal dementia (FTD).

In a preferred embodiment of the invention said neurodegenerative disease is Fragile X-associated tremor/ataxia syndrome (FXTAS).

According to an aspect of the invention there is provided a cell penetrating polypeptide comprising or consisting of an amino acid sequence set forth in SEQ ID NO 90.

In a preferred embodiment of the invention said polypeptide is between 12-42 or preferably between 13-42 amino acids in length.

In a further preferred embodiment of the invention said polypeptide comprises or consist of an amino acid sequence set forth in SEQ ID NO 75.

According to an aspect of the invention there is provided a polypeptide according to the invention for use as a medicament.

According to a further aspect of the invention there is provided a polypeptide according to the invention for use in the treatment of a neurodegenerative disease.

In a preferred embodiment of the invention said neurodegenerative disease is selected from the group consisting of: amyotrophic lateral sclerosis (ALS) sporadic amyotrophic lateral sclerosis, familial ALS caused by a mutation other than a pathological C9ORF72-repeat expansion, frontotemporal dementia (FTD) motor neurone disease, frontotemporal lobar dementia (FTLD), Huntington's like disorder, and Fragile X-associated tremor/ataxia syndrome (FXTAS).

In a preferred embodiment of the invention said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

In a preferred embodiment of the invention said neurodegenerative disease is sporadic and/or familial amyotrophic lateral sclerosis.

In a preferred embodiment of the invention said neurodegenerative disease is ALS not caused by pathological C9ORF72-repeat expansions

In a preferred embodiment of the invention said neurodegenerative disease is sporadic frontotemporal dementia (FTD).

In a preferred embodiment of the invention said neurodegenerative disease is Fragile X-associated tremor/ataxia syndrome (FXTAS).

According to a further aspect of the invention there is provided an antagonistic agent comprising a nucleic acid molecule wherein said nucleic acid molecule comprises a

nucleotide sequence designed with reference to human Serine/Arginine Rich Splice Factor (SRSF1) and wherein said nucleic acid molecule inhibits expression of SRSF1.

In a preferred embodiment of the invention said nucleic acid molecule is a double stranded nucleic acid molecule comprises a sense strand and an antisense strand comprising a nucleotide sequence wherein said antisense nucleotide strand is adapted to anneal by complementary base pairing to a nucleic acid molecule encoding human SRSF1.

In a preferred embodiment of the invention said double stranded nucleic acid molecule is RNA. Preferably, said RNA is siRNA or miRNA.

In an alternative embodiment of the invention said nucleic acid molecule is a single stranded nucleotide sequence comprising an antisense nucleotide sequence wherein said antisense nucleotide sequence is adapted to anneal by complementary base pairing to a nucleic acid molecule encoding SRSF1.

In a preferred embodiment of the invention said single stranded nucleic acid is DNA.

In a further preferred embodiment of the invention said single stranded nucleic acid is DNA and/or RNA.

Preferably, said DNA and/or RNA is a therapeutic antisense oligonucleotide such as an antisense oligonucleotide, a splice-switching oligonucleotide, a gapmer or similar.

Preferably said DNA is an antisense oligonucleotide.

In a preferred embodiment of the invention said nucleic acid molecule encoding human SRSF1 is set forth in SEQ ID NO: 67.

In a preferred embodiment of the invention said antagonistic agent comprises a nucleic acid molecule that is at least 15 nucleotides in length.

In a preferred embodiment of the invention said antagonistic agent comprises a nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID NO: 67 wherein said nucleic acid molecule is a double stranded inhibitory RNA and is 19-23 nucleotides in length.

In a preferred embodiment of the invention said antagonistic agent comprises a nucleic acid molecule comprises modified nucleotides.

In a preferred embodiment of the invention said double stranded nucleic acid molecule comprising sense and antisense nucleic acid molecules comprise modified nucleotides.

In a preferred embodiment of the invention said modified nucleotides/sugars are selected from the group: a 3 '-terminal deoxy- thymine (dT) nucleotide, a 2'-0-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-0-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2' -hydroxly- modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-0- alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising phosphorodithioate (PS2), a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'- phosphate, and a nucleotide comprising a 5 '-phosphate mimic, for example a 5'-vinyl phosphate, a nucleotide comprising a 2'-deoxy-2'-fluro and a 2' methyl sugar base.

In a preferred embodiment of the invention said double stranded nucleic acid molecule comprising sense and antisense nucleic acid molecules comprise modified sugar(s).

In a preferred embodiment of the invention said modified sugar is selected from the group: a modified version of the ribosyl moiety, such as -O- modified RNA such as 2'-O-alkyl or 2'-O-(substituted)alkyl e.g. 2'-0-methyl, T-0-(2- cyanoethyl), 2'-0-(2-methoxy)ethyl (2'-MOE), 2'-0-(2-thiomethyl)ethyl, 2'-O-butyryl, -O- propargyl, 2'-O-allyl, 2'-O-(2-amino)propyl, 2'-O-(2-(dimethylamino)propyl), 2'-O-(2- amino)ethyl, 2'-O-(2-(dimethylamino)ethyl); 2'-deoxy (DNA); 2'-O-(haloalkoxy)methyl, e.g. 2'-0-(2-chloroethoxy)methyl (MCEM), -O- (2,2-dichloroethoxy)methyl (DCEM); 2'-<3-alkoxycarbonyl e.g. T-0-[2- (methoxycarbonyl)ethyl] (MOCE), 2'-O-[2-(N-methylcarbamoyl)ethyl] (MCE), T-0-[2-(N,N- dimethylcarbamoyl)ethyl] (DCME); 2'-halo e.g. 2'-F, FANA (2'-F arabinosyl nucleic acid); carbasugar and azasugar modifications; 3 '-O-alkyl e.g. 3'-0-methyl, 3 '-0-butyryl, V-O- propargyl and their derivatives.

In a preferred embodiment of the invention said antagonistic agent comprises or consists of a nucleotide sequence designed with reference to the target nucleic acid sequences selected from the group:

TGGCACTGGTGTCGTGGAGTTTGTA (SEQ ID NO 110);
TGGTGTCGTGGAGTTTGTACGGAAA (SEQ ID NO 111);
TCGTGGAGTTTGTACGGAAAGAAGA (SEQ ID NO 112);
AAGATATGACCTATGCAGTTCGAAA (SEQ ID NO 113);
GAGAAACTGCCTACATCCGGGTTAA (SEQ ID NO 114);
CGGGTTAAAGTTGATGGGCCCAGAA (SEQ ID NO 115);
TGATGGGCCCAGAAGTCCAAGTTAT (SEQ ID NO 116);
CAGAAGTCCAAGTTATGGAAGATCT (SEQ ID NO 117);
GAGAAGCAGAGGATCACCACGCTAT (SEQ ID NO 118); and
CGTCATAGCAGATCTCGCTCTCGTA (SEQ ID NO 119).

In a preferred embodiment of the invention said antagonistic agent comprises a nucleic acid molecule comprising a nucleotide sequence wherein said nucleic acid molecule is a double stranded inhibitory RNA and is 19-23 nucleotides in length.

According to a further aspect of the invention there is provided a pharmaceutical composition comprising an antagonist agent according to the invention according and including an excipient or carrier.

According to a further aspect of the inventio there is provided an antagonistic agent according to the invention for use as a medicament.

According to a further aspect of the invention there is provided an antagonistic agent to the invention for use in the treatment of a neurodegenerative disease.

In a preferred embodiment of the invention said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

In a preferred embodiment of the invention said neurodegenerative disease is sporadic and/or familial amyotrophic lateral sclerosis.

In a preferred embodiment of the invention said neurodegenerative disease is ALS not caused by pathological C9ORF72-repeat expansion.

In an alternative preferred embodiment of the invention said neurodegenerative disease is sporadic frontotemporal dementia (FTD).

In an alternative preferred embodiment of the invention said neurodegenerative disease is Fragile X-associated tremor/ataxia syndrome (FXTAS).

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", means "including but not limited to", and is not intended to (and does not) exclude other moieties, additives, components, integers or steps. "Consisting essentially" means having the essential integers but including integers which do not materially affect the function of the essential integers.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

An embodiment of the invention will now be described by example only and with reference to the following figures:

Figure 1. Timeline for differentiation and co-culture of motor neurons and astrocytes derived from healthy control and sporadic ALS (sALS) patients;

Figure 2. Images show that MNs treated with lentivirus expressing SRSF1-miRNA retain processes/axons characteristic of neurons compared to MN treated with LV_Ctrl-miRNA which degenerate and die;

Figure 3. Bar charts show MN survival expressed as a ratio of MNs quantified at counting day 3 over day 1 (%). 2-way ANOVA with Tukey's multiple comparison test; NS: non-significant; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$;

Figure 4 Western immunoblotting shows that all 3 shRNAs lead to efficient depletion of SRSF1 and inhibition of the RAN translation of V5-tagged DPRs;

Figure 5. Bar charts represents mean±sem (2-way ANOVA with Tukey's multiple comparison test; NS: non-significant; ****: $p<0.0001$; n=3 biological replicates). Quantification in 3 independent triplicate experiments;

Figure 6. C9ORF72-ALS/FTD mice were injected via cisterna magna at post-natal day 1 (P1) with either 8 x 10^{10} scAAV9_Ctrl-shRNA_GFP vector genomes (vg) or 6 x 10^{10} scAAV9_SRSF1-shRNA10_GFP vg. Animals were sacrificed 1 month and 3 months post injections. Western blots show that the scAAV9_SRSF1-shRNA10_GFP virus leads to specific depletion of SRSF1 in C9ORF72-ALS/FTD mice as well as in wild type C57BL/6 mice (not shown) while the Ctrl-shRNA has no effect. GAPDH is used as a loading control;

Figure 7: map of scAAV_SRSF1 132-144 CPP_GFP (SEQ ID NO 1 and 101);

Figure 8: map of scAAV_SRSF1 89-120 CPP_GFP (SEQ ID NO 74 and 102);

Figure 9: (A) Western blots show depletion of SRSF1 and inhibition of the RAN translation of sense DPRs upon co-transfection with scAAV SRSF1-shRNA10_GFP, H1-CPP1_GFP and H1-CPP2_GFP, but not when CPPs transcription is driven the RNAPII promoter. SRSF1 and DPRs expression levels are quantified in triplicate biological experiments in panels B and C respectively. (D) MTT cell proliferation assays in biological triplicates showing that scAAV SRSF1-shRNA10_GFP, H1-CPP1_GFP and H1-CPP2_GFP alleviates the cytotoxicity mediated by the expression of DPRs, but not when CPPs transcription is driven the RNAPII promoter;

Figure 10: (A) Western blots show depletion of SRSF1 and inhibition of the RAN translation of antisense DPRs upon co-transfection with scAAV SRSF1-shRNA10_GFP, H1-CPP1_GFP and H1-CPP2_GFP, but not when CPPs transcription is driven the RNAPII promoter. SRSF1 and DPRs expression levels are quantified in triplicate biological experiments in panels B and C respectively. (D) MTT cell proliferation assays in biological triplicates showing that scAAV SRSF1-shRNA10_GFP, H1-CPP1_GFP and H1-CPP2_GFP alleviates the cytotoxicity mediated by the expression of DPRs, but not when CPPs transcription is driven the RNAPII promoter;

Figure 11 map of scAAV_SRSF1 -shRNA10_GFP (SEQ ID NO 66 and 103);

Figure 12 DPR quantification in mouse brains. C9ORF72-ALS/FTD (C9-Tg) mice were injected intrathecally (via cisterna magna) with $6x10^{10}$ vector genome (vg) of scAVV9_Ctrl-shRNA_GFP or 2 doses of of scAAV9_SRSF1-shRNA10_GFP ($4x10^{10}$ and $8x10^{10}$ vg) at post-natal day 1-2 (P1-2). Non-transgenic (NTg) mice are used as a control. Animals were sacrificed 1 month **(A)** or 3 months **(B)** post injection prior to MSD- ELISA quantification of poly-GP DPRs in the cerebellum/brainstem (mean ± SEM; one-way ANOVA with Tukey's correction for multiple comparisons; NS: non-significant, **: $p<0.01$, ****: $p<0.0001$; n=4-6 mice/group). Poly-GP DPRs were quantified against a standard curve established with a GPx7 peptide and levels normalised to 100 % for the untreated C9-Tg mice;

Figure 13 Viral transduction in mouse brains. Immunohistochemical analysis of C9ORF72-ALS/FTD mice injected intrathecally (via cisterna magna) with 5x1010 vector genome (vg) of scAAV9_H1-SRSF1-CPP1_GFP or scAAV9_H1-CPP2_GFP at post-natal day 1-2 (P1-2). Animals were sacrificed one month post injection prior to anti-GFP immunofluorescence microscopy in the brain. Representative images are shown on the sections of midbrain. GFP co-expression is displayed in the green channel. DAPI (blue channel) and NeuN (red channel) stain nuclei and and mature neurons respectively. Side panels: Enlarged immunofluorescence images showing transduction and scAAV9-mediated co-expression of of GFP expression in both neuronal and microglial cells. Scale bars represent 500 µm; and

Figure 14 Viral biodistribution and DPR quantification in mouse brains. C9ORF72-ALS/FTD (C9-Tg) mice were injected intrathecally (via cisterna magna) with 5x1010 vector genome (vg) of scAAV9_H1-SRSF1-CPP1_GFP or scAAV9_H1-CPP2_GFP at post-natal day 1-2 (P1-2). Non-transgenic (NTg) mice are used as a control. Animals were sacrificed one month post injection. (A) qPCR quantification of viral DNA extracted from the brain (cerebellum) and spinal cords (n=3), showing efficient transduction. (B) MSD- ELISA quantification of poly-GP DPRs in the cerebellum/brainstem (mean ± SEM; one-way ANOVA with Tukey's correction for multiple comparisons; **: $p<0.01$, ****: $p<0.0001$; N=3 mice/group). Poly-GP DPRs were quantified against a standard curve established with a GPx7 peptide and levels normalised to 100 % for the untreated C9-Tg mice.

Materials and Methods

PART 1: SRSF1 depletion promotes the survival of sALS patient-derived motor neurons co-cultured with astrocytes

1/ Timeline for differentiation and co-culture of motor neurons and astrocytes derived from healthy control and sporadic ALS (sALS) patients:

Summary: Both iMotor Neurons (iMNs) and iAstrocytes are treated with either 5 MOI (Multiplicity of Infection) of lentivirus (LV) expressing a Ctrl-miRNA or 2 chained miRNAs directed against SRSF1 (constructs described in Hautbergue et al, Nature Communications 2017; 8:16063 and in our patent WO2017207979A1) at day 18 and 3 of the differentiation respectively, prior to establishing co-culture from day 20 (iMN) / 5 (iA). High content automated live imaging quantify iMN survival at day 22, 23, 24. scAAV9 does not efficiently transduce cells *in vitro*, in contrast to lentivirus which have been used here in this system.

Detailed protocol: Co-cultures of patient-derived astrocytes and motor neurons

Differentiation of iMotor Neurons (iMNs). Human patient and control-derived neurons (iNeurons) were differentiated from induced neural progenitor cells (iNPCs) using a modified version of protocol (Meyer K et al. Proc. Natl. Acad. Sci. U.S.A. 2014; 111:829–832) as previously described (Hautbergue GM et al, Nature Communications 2017; 8:16063). In brief, 100,000 iNPCs were plated in a 6-well plate coated with fibronectin (Millipore) and expanded to 70-80% confluence. Once they reached this confluence, iNPC medium was replaced with neuron differentiation medium (DMEM/F-12 with glutamax supplemented with 1% N2, 2% B27 (Gibco) containing 2.5 µM of DAPT (Tocris) to determine differentiation towards neuronal lineage on day 1. On day 3, the neuron differentiation medium was supplemented with 1 µM retinoic acid (Sigma), 0.5 µM smoothened agonist (SAG) (Millipore) and 2.5 µM forskolin (Sigma) for 7 days until Day 10. This protocol leads to typical yields of 70% β-III tubulin (Tuj1) positive cells. To obtain iMotor Neurons (iMN), ~ 5,000 iNeurons per well were re-plated on 96-well plates coated with fibronectin and maintained in iNeuron differentiation medium (containing retinoic acid, SAG and forskolin) supplemented with BDNF, CNTF and GDNF (all at 20 ng/ml) for the last 14 days of differentiation.

Differentiation of iAstrocytes. Human patient-derived astrocytes (iAstrocytes) were differentiated from iNPCs as previously described (Meyer K et al. Proc. Natl. Acad. Sci. U.S.A. 2014; 111:829–832; Hautbergue GM et al, Nature Communications 2017; 8:16063) and cultured in DMEM glutamax (Gibco) with 10% FBS (Sigma) and 0.02% N2 (Invitrogen) for 5 days. Cells were maintained in a 37°C incubator with 5% CO2.

Co-cultures of patient-derived iMNs and iAstrocytes. iAstrocytes were lifted at day 5 of differentiation and ~5,000 iAstrocytes were re-plated on iMNs at day 20 of differentiation. Co-cultured iMNs and iAstrocytes were maintained in neuron differentiation medium with BDNF,

GDNF and CTNF (all at 20 ng/ml) for 4 days. 12 h after the start of co-cultures (on day 21), 1 or 10 µM CPP was added to the medium and iMNs/ iAstrocytes were imaged for 72 h at days 22, 23, 24. For SRSF1 knockdown, iMNs and iAstrocytes were separately transduced 48h prior to co-culture with lentivirus (LV) expressing control or SRSF1-RNAi co-expressing GFP (Hautbergue GM et al, Nature Communications 2017; 8:16063) at a MOI of 5 at day 18 of iMN differentiation and at day 3 of iAstrocyte differentiation.

PART 2: scAAV9-driven expression of SRSF1-shRNA

Pre-clinical vector design: scAAV_SRSF1-shRNA_GFP

1/ SRSF1-shRNA cassette targeting mouse, rat, non-human primate and human SRSF1

Take region human SRSF1 448-750 (3' end of open reading frame) which is highly conserved with mouse SRSF1.

Human SRSF1 (NM_006924.4) SEQ ID NO 67

gctgatgtttaccgagatggcactggtgtcgtggagtttgtacggaaagaagatatgacctatgcagttcgaaaactggataacac taagtttagatctcatgagggagaaactgcctacatccgggttaaagttgatgggcccagaagtccaagttatggaagatctcgat ctcgaagccgtagtcgtagcagaagccgtagcagaagcaacagcaggagtcgcagttactccccaaggagaagcagagga tcaccacgctattctccccgtcatagcagatctcgctctcgtacataa

ttaaagttgatgggcccagaa miRNA (SEQ ID NO 87) used to target human and mouse SRSF1 in the lentivirus construct (Hautbergue et al. Nature Communications 2017; 8:16063 and in our patent WO2017207979A1)

Design shRNA using the following website:

Block-iT RNAi Designer tool: http://rnaidesigner.lifetechnologies.com/rnaiexpress/

Table 1: SEQ ID NO 2-11

No.	Start (nt)	Target sequence (DNA)	Region	GC%	Rank (predicted efficacy 0-5)
2	1	GCTGATGTTTACCGAGATGGC	52.39		3.5
3	33	GGAGTTTGTACGGAAAGAAGA	42.86		4.5
4	44	GGAAAGAAGATATGACCTATG not fully conserved human/mouse	38.1		3.5
5	45	GAAAGAAGATATGACCTATGC not fully conserved human/mouse	38.1		3.5
6	115	GCCTACATCCGGGTTAAAGTT	47.62		3.5

7	139	GGGCCCAGAAGTCCAAGTTAT	52.39	4.5
8	140	GGCCCAGAAGTCCAAGTTATG	52.39	3.5
9	141	GCCCAGAAGTCCAAGTTATGG	52.39	4.0
10	160	GGAAGATCTCGATCTCGAAGC	52.39	4.5
11	245	GCAGAGGATCACCACGCTATT	52.39	5.0

Table 2: Use siSPOTR (Boudreau RL et al. Nucleic Acids Res. 2013;41(1):e9) to predict the off target of the common human/mouse sequences targeting SRSF1 and predicted most efficient

shRNA sequence	antisense/ mature shRNA sequence	POTS	POTS	Seed
77	gccaucucgguaaacaucagc	355.351 (mouse)	463.716 (human)	CCATCTC
78	ucuucuuuccguacaaacucc	501.411 (mouse)	588.488 (human)	CTTCTTT
79	cauaggucauaucuucuuucc	96.137 (mouse)	149.126 (human)	ATAGGTC
80	gcauaggucauaucuucuuuc	140.373 (mouse)	167.649 (human)	CATAGGT
81	aacuuuaacccggauguaggc	390.256 (mouse)	526.984 (human)	ACTTTAA
82	auaacuuggacuucugggccc	221.397 (mouse)	339.052 (human)	TAACTTG
83	cauaacuuggacuucugggcc	237.138 (mouse)	351.458 (human)	ATAACTT
84	ccauaacuuggacuucugggc	159.58 (mouse)	215.339 (human)	CATAACT
85	gcuucgagaucgagaucuucc	41.326 (mouse)	41.3938 (human)	CTTCGAG
86	aauagcguggugauccucugc	21.324 (mouse)	22.5396 (human)	ATAGCGT

SRSF1 target shRNA6 sequence: 5'- GGGCCCAGAAGTCCAAGTTAT -3' (SEQ ID NO 7)
Antisense/mature shRNA6 sequence: 5'- AUAACUUGGACUUCUGGGCCC -3' (SEQ ID NO 82)

SRSF1 target shRNA9 sequence: 5'- GGAAGATCTCGATCTCGAAGC -3' (SEQ ID NO 10)
Antisense/mature shRNA9 sequence: 5'- GCUUCGAGAUCGAGAUCUUCC -3'(SEQ ID NO 85)

SRSF1 target shRNA10 sequence: 5'- GCAGAGGATCACCACGCTATT -3' (SEQ ID NO 11)
Antisense/mature shRNA10 sequence: 5'- AAUAGCGUGGUGAUCCUCUGC -3' (SEQ ID NO 86)

2/ Alignment human (NM_006924.4; SEQ ID NO 104) and mouse (NM_173374.4; SEQ ID NO 105) SRSF1

Sequences corresponding to the shRNAs 7, 10 and 11 (predicted the most efficient with the less predicted off-target effects) are highlighted on the aligned human and mouse SRSF1 open reading frames.

```
hSRSF1   ATGTCGGGAGGTGGTGTGATTCGTGGCCCCGCAGGGAACAACGATTGCCGCATCTACGTG  60
         |||||||||||||||||||| ||||||||  || |||||||||| ||||||||||||||||
mSRSF1   ATGTCGGGAGGTGGTGTGATCCGTGGCCCGGCGGGGAACAACGACTGCCGCATCTACGTG  60

hSRSF1   GGTAACTTACCTCCAGACATCCGAACCAAGGACATTGAGGACGTGTTCTACAAATACGGC  120
         ||||||  |||||||  ||  ||||||||||||||||  ||||||||||  ||||||||||
mSRSF1   GGTAACCTACCTCCGGATATCCGAACCAAGGACATCGAGGACGTGTTTTACAAATACGGC  120

hSRSF1   GCTATCCGCGACATCGACCTCAAGAATCGCCGCGGGGGACCGCCCTTCGCCTTCGTTGAG  180
         ||  ||||||||||||||||  |||||  ||||||||||||||||||||||||||||||||
mSRSF1   GCCATCCGCGACATCGACCTGAAGAACCGCCGCGGGGGACCGCCCTTCGCCTTCGTTGAG  180

hSRSF1   TTCGAGGACCCGCGAGACGCGGAAGACGCGGTGTATGGTCGCGACGGCTATGATTACGAT  240
         |||||||||||||||||||||||||  ||||||||  ||||||||||||||  ||  |||||
mSRSF1   TTCGAGGACCCGCGAGACGCGGAAGATGCGGTGTACGGTCGCGACGGCTACGACTACGAC  240

hSRSF1   GGGTACCGTCTGCGGGTGGAGTTTCCTCGAAGCGGCCGTGGAACAGGCCGAGGCGGCGGC  300
         ||  |||||  ||||||||  ||||||||  ||||||||||  ||  ||  ||||||||||||||||
mSRSF1   GGCTACCGGCTGCGGGTAGAGTTTCCCCGAAGCGGCCGCGGGACCGGCCGAGGCGGCGGC  300

hSRSF1   GGGGGTGGAGGTGGCGGAGCTCCCCGAGGTCGCTATGGCCCCCCATCCAGGCGGTCTGAA  360
         ||||||||||  |||||  ||  ||  ||||  ||||||||||  ||  ||||||||||  ||
mSRSF1   GGGGGTGGAGGCGGCGGCGCCCCGAGAGGCCGCTATGGCCCGCCGTCCAGGCGGTCCGAG  360

hSRSF1   AACAGAGTGGTTGTCTCTGGACTGCCTCCAAGTGGAAGTTGGCAGGATTTAAAGGATCAC  420
         ||||||||||||||||||||||||||||||  ||||||||  ||||||||  ||||||||||||
mSRSF1   AACAGAGTGGTTGTCTCTGGACTGCCTCCGAGTGGAAGCTGGCAGGACTTAAAGGATCAC  420

hSRSF1   ATGCGTGAAGCAGGTGATGTATGTTATGCTGATGTTTACCGAGATGGCACTGGTGTCGTG  480
         ||||||||  ||||||||||||||||||  ||||||||||||||||||||||||||||||||
mSRSF1   ATGCGTGAGGCAGGTGATGTATGTTACGCTGATGTTTACCGAGATGGCACTGGTGTCGTG  480

hSRSF1   GAGTTTGTACGGAAAGAAGATATGACCTATGCAGTTCGAAAACTGGATAACACTAAGTTT  540
         |||||||||||||||||||||||||  ||||||||||||||||||||||||||||||||||
mSRSF1   GAGTTTGTACGGAAAGAAGATATGACGTATGCAGTTCGAAAACTGGATAACACTAAGTTT  540

hSRSF1   AGATCTCATGAGGGAGAAACTGCCTACATCCGGGTTAAAGTTGATGGGCCCAGAAGTCCA  600
         ||||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||||
mSRSF1   AGATCTCACGAGGGAGAAACTGCCTACATCCGGGTTAAAGTTGATGGGCCCAGAAGTCCA  600
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hSRSF1   AGTTATGGAAGATCTCGATCTCGAAGCCGTAGTCGTAGCAGAAGCCGTAGCAGAAGCAAC  660
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
mSRSF1   AGTTATGGAAGATCTCGATCTCGAAGCCGTAGTCGTAGCAGAAGCCGTAGCAGAAGCAAC  660

hSRSF1   AGCAGGAGTCGCAGTTACTCCCCAAGGAGAAGCAGAGGATCACCACGCTATTCTCCCCGT  720
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
mSRSF1   AGCAGGAGTCGCAGTTACTCCCCAAGGAGAAGCAGAGGATCACCACGCTATTCTCCCCGT  720

hSRSF1   CATAGCAGATCTCGCTCTCGTACATAA  747
         ||||||||||||||||||||||||||
mSRSF1   CATAGCAGATCTCGCTCTCGTACATAA  747
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shRNA	antisense/ mature shRNA sequence	POTS	POTS	Seed sequence
82	auaacuuggacuucugggccc	221.397 (mouse)	339.052 (human)	TAACTTG
86	gcuucgagaucgagaucuucc	41.326 (mouse)	41.3938 (human)	CTTCGAG
96	aauagcguggugauccucugc	21.324 (mouse)	22.5396 (human)	ATAGCGT

3/ Cloning of SRSF1-targeting shRNAs into the scAAV_GFP vector

We then designed and custom synthesised the following oligonucleotides for cloning shRNAs 7, 10 and 11 into our scAAV_H1promoter_GFP vector (SEQ ID NO 68-73):

Cut BamHI / cut HindIII Red sequences corresponds to SRSF1 targeted region Blue sequences correspond to antisense/mature shRNA Black sequence corresponds to hairpin loop

SRSF1 shRNA_6_fwd (SEQ ID NO 68)

GATCC GGGCCCAGAAGTCCAAGTTAT TTCAAGAGA ATAACTTGGACTTCTGGGCCC C TTTTTT GGA A

SRSF1 shRNA_6_rev (SEQ ID NO 69):

AGCTT TCC AAAAAA G GGGCCCAGAAGTCCAAGTTAT TCTCTTGAA ATAACTTGGACTTCTGGGCCC G

SRSF1 shRNA_9_fwd (SEQ ID NO 70):

GATCC GGAAGATCTCGATCTCGAAGC TTCAAGAGA GCTTCGAGATCGAGATCTTCC C TTTTTT GGA A

SRSF1 shRNA_9_rev (SEQ ID NO 71):

AGCTT TCC AAAAAA G GGAAGATCTCGATCTCGAAGC TCTCTTGAA GCTTCGAGATCGAGATCTTCC G

SRSF1 shRNA_10_fwd (SEQ ID NO 72):

GATCC GCAGAGGATCACCACGCTATT TTCAAGAGA AATAGCGTGGTGATCCTCTGC C TTTTTT GGA A

SRSF1 shRNA_10_rev (SEQ ID NO 73):

AGCTT TCC AAAAAA G GCAGAGGATCACCACGCTATT TCTCTTGAA AATAGCGTGGTGATCCTCTGC G

5/ Full sequence of the pre-clinical scAAV vector co-expressing the SRSF1-shRNA cassette (under constitutively-expressed RNAPIII H1 promoter) and eGFP (under a weak RNAPII eF-1alpha core promoter to avoid potential GFP-induced toxicity)

scAAV_SRSF1-shRNA10_GFP circular sequence (5,648 bp) SEQ ID NO 66

5'...

GCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCAC
TCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCA
GCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAG
CATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCC
CCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCC
CTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCC
AAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGA
GTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGT
ATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTA
TCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG
CTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTT
TGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTAT
CAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAA
CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT
AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAA
TGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGA
GCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAG
TAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTT
TGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAA
AGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTAT
GGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACC
AAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGC
GCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTT
ACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACC
AGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATG
TTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACA
TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGT
CTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGT
TTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGA
TGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTA
TGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATAGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATG
CGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGA
AAATACCGCATCAGGCGATTCCAACATCCAATAAATCATACAGGCAAGGCAAAGAATTAGCAAAATTAAGCAA
TAAAGCCTCAGAGCATAAAGCTAAATCGGTTGTACCAAAAACATTATGACCCTGTAATACTTTTGCGGGAGAA
GCCTTTATTTCAACGCAAGGATAAAAATTTTTAGAACCCTCATATATTTTAAATGCAATGCCTGAGTAATGTGT

AGGTAAAGATTCAAACGGGTGAGAAAGGCCGGAGACAGTCAAATCACCATCAATATGATATTCAACCGTTCT
AGCTGATAAATTCATGCCGGAGAGGGTAGCTATTTTTGAGAGGTCTCTACAAAGGCTATCAGGTCATTGCCT
GAGAGTCTGGAGCAAACAAGAGAATCGCCGGGGGGGGGGGGGGGGGGGGCCACTCCCTCTCTGCGCGCT
CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTG
AGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTCAGATCGATCTCTCCCCA
GCATGCAGGCCTCTGCAGTCGACGGGCCCGGCATGCGTTTTACTCCCCAGCATGCCTGCTATTCTCTTCCC
AATCCTCCCCCTTGCTGTCCTGCCCCACCCCACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATG
CAATTTCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAG
GGGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGCTGATCAGCGAGCTCTAGGAATTTTACT
TGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATCGCG
CTTCTCGTTGGGGTCTTTGCTCAGGGCGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGG
CCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCCTCGATGTTGTGGC
GGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGTCGGCCATGATATAGACGTTGTGGCTGTTGTAGTT
GTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTCA
CCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTG
CGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGC
GGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGG
TGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAA
CTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCC
TTGCTCACCATGCCTGTGTTCTGGCGGCAAACCCGTTGCGAAAAAGAACGTTCACGGCGACTACTGCACTTA
TATACGGTTCTCCCCCACCCTCGGGAAAAAGGCGGAGCCAGTACACGACATCACTTTCCCAGTTTACCCCG
CGCCACCTTCTCTAGGCACCGGATCAATTGCCGACCCCTCCCCCCAACTTCTCGGGGACTGTGGGCGATGT
GCGCTCTGCCCACTGAATCTTCTCGAGCCTCTAGATACCACAGGCTGCGCAACTGTTGGGAAGGGCGATCG
GTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAA
CGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCATATTTGCATGTCGCTATGTGT
TCTGGGAAATCACCATAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTCGG
ATCCGCAGAGGATCACCACGCTATTTTCAAGAGAAATAGCGTGGTGATCCTCTGCCTTTTTTGGAAAGCTTG
GCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCG
GAAGCATAAAGTGTATCTAGAGCGGTACCACGCGTGAATTGAATTCAGATCCACGCGTGAATTCCACTCCCT
CTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGG
CGGCCTCAGTGAGCGAGCGAGCGCGCAGGGCGATGAACGGTAATCGTAAAACTAGCATGTCAATCATATGT
ACCCCGGTTGATAATCAGAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAGCGTTA
ATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAA
ATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTAT
TAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCA
TCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCG
ATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGG
CGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCG
CTACAGGGCGCGTACTATGGTTGCTTTGACGAGCACGTATAACGTGCTTTCCTCGTTAGAATCAGAGCGGG
AGCTAAACAGGAGGCCGATTAAAGGGATTTTAGACAGGAACGGTACGCCAGAATCCTGAGAAGTGTTTTTAT
AATCAGTGAGGCCACCGAGTAAAAGAGTCTGTCCATCACGCAAATTAACCGTTGTCGCAATACTTCTTTGATT
AGTAATAACATCACTTGCCTGAGTAGAAGAACTCAAACTATCGGCCTTGCTGGTAATATCCAGAACAATATTA
CCGCCAGCCATTGCAACGGAATCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTG
CGGGCCTCTTCGCTATTACGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTAT
TGGGC... 3’

6/ Functionality of scAAV9-driven expression of SRSF1-shRNA10 in mouse brains.

C9ORF72-ALS/FTD mice were injected via cisterna magna at post-natal day 1 (P1) with either 8 x 10^{10} scAAV9_Ctrl-shRNA_GFP vector genomes (vg) or 6 x 10^{10} scAAV9_SRSF1-shRNA10_GFP vg. Animals were sacrificed 1 month and 3 months post injections. Western blots show that the scAAV9_SRSF1-shRNA10_GFP virus leads to specific depletion of SRSF1 in C9ORF72-ALS/FTD mice as well as in whild type C57Bl6 mice (not shown) while the Ctrl-shRNA has no effect. GAPDH is used as a loading control.

4/ ITR sequences

SEQ ID NO 64:

ITR1: 5'-ccactccctctctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccggcctcagtgagcgagcgagcg cgcagagagggagtggccaactccatcactaggggttcct -3'

SEQ ID NO 88:

ITR2: 5'-ccactccctctctgcgcgctcgctcgctcactgaggccgggcgaccaaaggtcgcccgacgcccgggctttgcccgggcggcctcagtgagcgagcgagcg cgcag -3'

Sequence of Cell Permeable Peptide

SRSF1 132-144 CPP nucleotide sequence:

5'-GGCAGCTGGCAGGATCTGAAAGATCATATGCGCGAAGCCGGCGGTGGGAAACCGATTCCCAACCCGCTGC TGGGCCTCGATAGCACCGGCGGATATGGTCGCAAAAAGCGCAGACAGCGCCGGAGG -3' (SEQ ID NO 89)

SRSF1 132-144 CPP sequence which corresponds to SRSF1 amino acids 132-144, a V5 tag and the protein transduction domain TAT amino acids 47-57: Nt-GSWQDLKDHMREAGGGKPIPNPLLGLDSTGGYGRKKRRQRRR – Ct (SEQ ID NO 90)

5/ Sequence of the scAAV_SRSF1 89-120 CPP_GFP circular sequence (5,692 bp) (SEQ ID NO 74)

5'...GCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCAC TCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCA GCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAG CATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCC CCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCC

CTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCC
AAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGA
GTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGT
ATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTA
TCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG
CTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTT
TGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTAT
CAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAA
CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT
AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAA
TGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGA
GCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAG
TAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTT
TGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAA
AGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTAT
GGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACC
AAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGC
GCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTT
ACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACC
AGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATG
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TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGT
CTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGT
TTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGA
TGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTA
TGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGA
GAAAATACCGCATCAGGCGATTCCAACATCCAATAAATCATACAGGCAAGGCAAAGAATTAGCAAAATTAAG
CAATAAAGCCTCAGAGCATAAAGCTAAATCGGTTGTACCAAAAACATTATGACCCTGTAATACTTTTGCGGGA
GAAGCCTTTATTTCAACGCAAGGATAAAAATTTTTAGAACCCTCATATATTTTAAATGCAATGCCTGAGTAATG
TGTAGGTAAAGATTCAAACGGGTGAGAAAGGCCGGAGACAGTCAAATCACCATCAATATGATATTCAACCGT
TCTAGCTGATAAATTCATGCCGGAGAGGGTAGCTATTTTTGAGAGGTCTCTACAAAGGCTATCAGGTCATTG
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CTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTCAGATCGATCTCTCCC
CAGCATGCGTTTTACCTCCCCAGCATGCCTGCTATTCTCTTCCCAATCCTCCCCCTTGCTGTCCTGCCCCAC
CCCACCCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGAC
AGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTA
GAAGGCACAGTCGAGGCTGATCAGCGAGCTCTAGGAATTTTACTTGTACAGCTCGTCCATGCCGAGAGTGA
TCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGC
GGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCTGCTG
GTAGTGGTCGGCGAGCTGCACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGT
TCTTCTGCTTGTCGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCCCAGGATGT
TGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACC
TCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCTTCGGGCAT
GGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTC

AGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGC
TTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCA
GCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATGCCTGTGTTCTGGCG
GCAAACCCGTTGCGAAAAAGAACGTTCACGGCGACTACTGCACTTATATACGGTTCTCCCCCACCCTCGGG
AAAAAGGCGGAGCCAGTACACGACATCACTTTCCCAGTTTACCCCGCGCCACCTTCTCTAGGCACCGGATC
AATTGCCGACCCCTCCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGCTCTGCCCACTGAATCTTCTCG
AGCCTCTAGATACCACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCC
AGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACG
TTGTAAAACGACGGCCAGTGAATTCATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAA
TGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTCGGATCCGAAGCCACCATGCCGCGCAGC
GGCCGCGGCACCGGCCGCGGTGGGGGCGGCGGTGGAGGTGGCGGAGCCCCGAGAGGCCGCTATGGACC
GCCCAGCCGCCGGAGCGAAGGCGGTGGGAAACCGATTCCCAACCCGCTGCTGGGCCTCGATAGCACCGG
CGGATATGGTCGCAAAAAGCGCAGACAGCGCCGGAGGTAATTTTTTAAGCTTGGCGTAATCATGGTCATAG
CTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTATCT
AGAGCGGTACCACGCGTGAATTGAATTCAGATCCACGCGTGAATTCCACTCCCTCTCTGCGCGCTCGCTCG
CTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAG
CGAGCGCGCAGGGCGATGAACGGTAATCGTAAAACTAGCATGTCAATCATATGTACCCCGGTTGATAATCA
GAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAGCGTTAATATTTTGTTAAAATTCG
CGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAA
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AACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTT
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GAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAA
GTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTACTA
TGGTTGCTTTGACGAGCACGTATAACGTGCTTTCCTCGTTAGAATCAGAGCGGGAGCTAAACAGGAGGCCG
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AGTAAAAGAGTCTGTCCATCACGCAAATTAACCGTTGTCGCAATACTTCTTTGATTAGTAATAACATCACTTG
CCTGAGTAGAAGAACTCAAACTATCGGCCTTGCTGGTAATATCCAGAACAATATTACCGCCAGCCATTGCAA
CGGAATCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATT
ACGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGC -3’

6/ 5/ Sequence of the scAAV_SRSF1 132-144 CPP_GFP circular sequence (5,651 bp) (SEQ ID NO 1)

5’…

GCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCAC
TCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCA
GCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAG
CATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCC
CCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCC
CTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCC
AAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGA
GTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGT
ATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTA
TCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG

CTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTT
TGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTAT
CAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAA
CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT
AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAA
TGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGA
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TGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAA
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AAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGC
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TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGT
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TTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGA
TGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTA
TGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGA
GAAAATACCGCATCAGGCGATTCCAACATCCAATAAATCATACAGGCAAGGCAAAGAATTAGCAAAATTAAG
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GAAGCCTTTATTTCAACGCAAGGATAAAAATTTTTAGAACCCTCATATATTTTAAATGCAATGCCTGAGTAATG
TGTAGGTAAAGATTCAAACGGGTGAGAAAGGCCGGAGACAGTCAAATCACCATCAATATGATATTCAACCGT
TCTAGCTGATAAATTCATGCCGGAGAGGGTAGCTATTTTTGAGAGGTCTCTACAAAGGCTATCAGGTCATTG
CCTGAGAGTCTGGAGCAAACAAGAGAATCGCCGGGGGGGGGGGGGGGGGGGCCACTCCCTCTCTGCGCG
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TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTCAGATCGATCTCTCCC
CAGCATGCGTTTTACCTCCCCAGCATGCCTGCTATTCTCTTCCCAATCCTCCCCCTTGCTGTCCTGCCCCAC
CCCACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGAC
AGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAACAACAGATGGCTGGCAACTA
GAAGGCACAGTCGAGGCTGATCAGCGAGCTCTAGGAATTTTACTTGTACAGCTCGTCCATGCCGAGAGTGA
TCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGC
GGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCTGCTG
GTAGTGGTCGGCGAGCTGCACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGT
TCTTCTGCTTGTCGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCCCAGGATGT
TGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACC
TCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCTTCGGGCAT
GGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTC
AGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGC
TTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCA
GCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATGCCTGTGTTCTGGCG
GCAAACCCGTTGCGAAAAAGAACGTTCACGGCGACTACTGCACTTATATACGGTTCTCCCCCACCCTCGGG
AAAAAGGCGGAGCCAGTACACGACATCACTTTCCCAGTTTACCCCGCGCCACCTTCTCTAGGCACCGGATC

AATTGCCGACCCCTCCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGCTCTGCCCACTGAATCTTCTCG
AGCCTCTAGATACCACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCC
AGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACG
TTGTAAAACGACGGCCAGTGAATTCATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAA
TGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTCGGATCCGTTTAGTGAACCGTCAGAAGCC
ACCATGGGCAGCTGGCAGGATCTGAAAGATCATATGCGCGAAGCCGGCGGTGGGAAACCGATTCCCAACC
CGCTGCTGGGCCTCGATAGCACCGGCGGATATGGTCGCAAAAAGCGCAGACAGCGCCGGAGGTAATTTTTT
AAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATA
CGAGCCGGAAGCATAAAGTGTATCTAGAGCGGTACCACGCGTGAATTGAATTCAGATCCACGCGTGAATTC
CACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTT
GCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGGGCGATGAACGGTAATCGTAAAACTAGCATGTCAA
TCATATGTACCCCGGTTGATAATCAGAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGT
AAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAAT
CGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAG
TCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTAC
GTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGA
GCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAG
GAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTA
ATGCGCCGCTACAGGGCGCGTACTATGGTTGCTTTGACGAGCACGTATAACGTGCTTTCCTCGTTAGAATCA
GAGCGGGAGCTAAACAGGAGGCCGATTAAAGGGATTTTAGACAGGAACGGTACGCCAGAATCCTGAGAAGT
GTTTTTATAATCAGTGAGGCCACCGAGTAAAAGAGTCTGTCCATCACGCAAATTAACCGTTGTCGCAATACTT
CTTTGATTAGTAATAACATCACTTGCCTGAGTAGAAGAACTCAAACTATCGGCCTTGCTGGTAATATCCAGAA
CAATATTACCGCCAGCCATTGCAACGGAATCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCG
ATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGT
TTGCGTATTGGGC -3'

Functionality of scAAV9-driven expression of SRSF1-shRNA10 in mouse brains.

C9ORF72-ALS/FTD mice were injected via cisterna magna at post-natal day 1 (P1) with either 8 x 10^{10} scAAV9_Ctrl-shRNA_GFP vector genomes (vg) or 6 x 10^{10} scAAV9_SRSF1-shRNA10_GFP vg. Animals were sacrificed 1 month and 3 months post injections. Western blots show that the scAAV9_SRSF1-shRNA10_GFP virus leads to specific depletion of SRSF1 in C9ORF72-ALS/FTD mice as well as in wild type C57Bl6 mice (not shown) while the Ctrl-shRNA has no effect. GAPDH is used as a loading control.

Example 1

2/ iMN imaging examples at day 24

High content automated imaging (Opera Phenix) was used to quantify surving MNs at Day 1, 2 and 3 of imaging. Images (Figure 2) show that MNs treated with lentivirus expressing

SRSF1-miRNA retain processes/axons characteristic of neurons compared to MN treated with LV_Ctrl-RNAi which generate and die.

3/ iMN quantification

Co-culture of healthy control and sALS patient-derived MN and astrocytes show that LV_SRSF1-RNAi specifically promotes sALS MN survival in levels comparable to the depletion of SRSF1 in C9ORF72-ALD patient-derived MNs (Hautbergue GM et al, Nature Communications 2017; 8:16063; Castelli et al. bioRxiv 2021.05.23.445325v2) Bar charts show MN survival expressed as a ratio of MNs quantified at counting day 3 over day 1 (%). 2-way ANOVA with Tukey's multiple comparison test; NS: non-significant; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$ (Figure 3)

Example 2

4/ Testing the functionality of SRSF1-shRNAs in human cells and mouse brains

scAAV plasmids co-expressing GFP and SRSF1 shRNA 6, 9 or 10 were co-transfected with either sense or antisense C9ORF72-repeat reporter constructs expressing V5-tagged sense or antisense dipeptide repeat proteins (DPRs) in all frames in a repeat-associated non-AUG (RAN) translation manner. Western immunoblotting shows that all 3 shRNAs lead to efficient depletion of SRSF1 and inhibition of the RAN translation of V5-tagged DPRs. SRSF1-shRNA10 was selected for viral production and further experiments in mice as it has the lowest POTS score and predicted genome-wide off-target effect in both mouse and human.

Example 3

scAAV SRSF1-shRNA, CPP1 and CPP2 inhibits the production of sense DPRs and rescue the DPR-associated cytotoxicity in a human cell model of C9ORF72-ALS/FTD. Human HEK293T cells were co-transfected with sense G4C2x45 C9ORF72-repeat plasmid expressing sense V5-tagged dipeptide-repeat protein (DPRs) in a RAN translation manner and scAAV plasmids expressing SRSF1-shRNA10 or 2 different cell permeable peptides (CPP1: SRSF1 aa89-120 CPP (SEQ ID NO 59) and CPP2: SRSF1 aa132-144 CPP (SEQ ID NO 75)). We tested potential expression under mammalian ubiquitous RNA polymerase II (CBh) or RNA polymerase III (H1) promoters. As shown in Figure 9 (A) Western blots show depletion of SRSF1 and inhibition of the RAN translation of sense DPRs upon co-transfection with scAAV SRSF1-shRNA10_GFP, H1-CPP1_GFP and H1-CPP2_GFP, but not when CPPs transcription is driven the RNAPII promoter. SRSF1 and DPRs expression levels are quantified in triplicate biological experiments in panels B and C respectively (Bar charts shows mean ± SEM; 1-way ANOVA; NS: non-significant, ****: $p < 0.0001$). (D) MTT cell proliferation assays in biological triplicates showing that scAAV SRSF1-shRNA10_GFP, H1-CPP1_GFP

and H1-CPP2_GFP alleviates the cytotoxicity mediated by the expression of DPRs, but not when CPPs transcription is driven the RNAPII promoter. Bar charts shows mean ± SEM; 1-way ANOVA; NS: non-significant, ****: $p < 0.0001$.

Example 4

scAAV SRSF1-shRNA, CPP1 and CPP2 inhibits the production of antisense DPRs and rescue the DPR-associated cytotoxicity in a human cell model of C9ORF72-ALS/FTD. Human HEK293T cells were co-transfected with antisense G2C4x43 C9ORF72-repeat plasmid expressing antisense V5-tagged dipeptide-repeat protein (DPRs) in a RAN translation manner and scAAV plasmids expressing SRSF1-shRNA10 or 2 different cell permeable peptides (CPP1: SRSF1 aa89-120 CPP (SEQ ID NO 59) and CPP2: SRSF1 aa132-144 CPP (SEQ ID NO 75)). We tested potential expression under mammalian ubiquitous RNA polymerase II (CBh) or RNA polymerase III (H1) promoters. Figure 10 (A) Western blots show depletion of SRSF1 and inhibition of the RAN translation of antisense DPRs upon co-transfection with scAAV SRSF1-shRNA10_GFP, H1-CPP1_GFP and H1-CPP2_GFP, but not when CPPs transcription is driven the RNAPII promoter. SRSF1 and DPRs expression levels are quantified in triplicate biological experiments in panels B and C respectively (Bar charts shows mean ± SEM; 1-way ANOVA; NS: non-significant, ****: $p < 0.0001$). Note that there is no expression of CPP1 or CPP2 from the protein-coding CBh promoter. (D) MTT cell proliferation assays in biological triplicates showing that scAAV SRSF1-shRNA10_GFP, H1-CPP1_GFP and H1-CPP2_GFP alleviates the cytotoxicity mediated by the expression of DPRs, but not when CPPs transcription is driven the RNAPII promoter. Bar charts shows mean ± SEM; 1-way ANOVA; NS: non-significant, ****: $p < 0.0001$.

Example 5

The data for SRSF1-shRNA (Figure 12) shows that the svAAV9-SRSF1-shRNA10 virus leads to inhibition of the DPRs in mouse brains and complements the data showing that it leads to SRSF1 depletion in mouse brains.

Example 6

The data shows that the scAAV9 virus expressing CPP1 or CPP2 and co-expressing GFP efficiently transduced neuronal and glial cells in mouse brains (Figure 13) and leads to DPR inhibition (Figure 14).

CLAIMS

1. A viral vector comprising a transcription cassette for the expression of a nucleic acid molecule in a mammalian host cell wherein said nucleic acid molecule is operably linked to a promoter adapted to express said nucleic acid molecule in said mammalian host cell characterised in that said vector comprises a non-expressed nucleotide sequence and wherein said nucleic acid molecule encodes an antagonistic agent that targets Serine/Arginine Rich Splice Factor (SRSF1).

2. The viral vector according to claim 1 wherein said SRSF1 comprises or consist of a sequence set forth in SEQ ID NO 67.

3. The viral vector according to claims 1 or 2 wherein said antagonistic agent is a nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide or peptide.

4. The viral vector according to claims 1 or 2 wherein said antagonistic agent is a nucleic acid-based agent.

5. The viral vector according to any one of claims 1-2 or 4 wherein said nucleic acid-based agent is an antisense nucleic acid, an inhibitory RNA, a shRNA or miRNA molecule that is complementary to and inhibits the expression of a nucleic acid encoding a Serin/Arginine Rich Splice Factor (SRSF1).

6. The viral vector according to claim 5 wherein said inhibitory RNA comprises or consists of a nucleotide sequence as set forth in SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 or 58.

7. The viral vector according to claim 5 wherein said shRNA comprises or consist of a nucleotide sequence selected from the group consisting of SEQ ID NO 2, 3, 4, 5, 6, 7, 8, 9,10 and 11.

8. The viral vector according to claim 7 wherein said shRNA comprises or consist of a nucleotide sequence set forth in SEQ ID NO 7.

9. The viral vector according to claim 7 wherein said shRNA comprises or consist of a nucleotide sequence set forth in SEQ ID NO 10.

10. The viral vector according to claim 7 wherein said shRNA comprises or consist of a nucleotide sequence set forth in SEQ ID NO 11.

11. The viral vector according to claim 3 wherein said peptide comprises an amino acid sequence that is at least 32 amino acids in length and comprises the amino acid sequence set forth in SEQ ID NO: 59.

12. The viral vector according to claim 3 wherein said peptide is a dominant negative protein comprising a modification of the amino acid sequence set forth in SEQ ID NO: 60 or 61.

13. The viral vector according to claim 3 wherein said modified protein comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 62 or 63.

14. The viral vector according to claim 3 wherein said peptide comprises an amino acid sequence that is at least 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40 or 42 amino acids in length and set forth in SEQ ID NO: 90.

15. The viral vector according to claims 3 or 14 wherein said peptide comprises an amino acid sequence that is set forth in SEQ ID NO: 75 (GSWQDLKDHMREA).

16. The viral vector according to any one of claims 1-3 and 11-15 wherein said viral vector comprises a RNA Pol III terminator.

17. The viral vector according to any one of claims 1-16 wherein said vector comprises inverted terminal repeat nucleotide sequences, and optionally wherein said ITR sequences are set forth in SEQ ID NO 64 or in SEQ ID NO 88.

18. The viral vector according to any one of claims 1-17 wherein said promoter is selected from the group consisting of H1 Polymerase III promoter, U6 promoter, U7 promoter or the mammalian 7SK promoter.

19. The viral vector according to claim 18 wherein said vector is a H1 Polymerase III promoter, and optionally is set forth in SEQ ID NO 65.

20. The viral vector according to any one of claims 1-19 wherein said viral based vector is an adeno-associated virus [AAV], and optionally a self-complementary adeno-associated virus (scAAV).

21. The viral vector according to claim 20 wherein said viral based vector is scAAV9 or scAAVrh10.

22. A pharmaceutical composition comprising a viral vector according to any one of claims 1-21 and an excipient or carrier.

23. A viral vector according to any one of claims 1-21 for use as a medicament.

24. A viral vector according to any one of claims 1-21 for use in the treatment of a neurodegenerative disease.

25. The viral vector for use according to claim 24 wherein said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

26. The viral vector for use according to claim 24 wherein said neurodegenerative disease is sporadic and/or familial amyotrophic lateral sclerosis .

27. The viral vector for use according to claim 24 wherein said neurodegenerative disease is ALS not caused by pathological C9ORF72-repeat expansions

28. The viral vector for use according to claim 24 wherein said neurodegenerative disease is sporadic frontotemporal dementia (FTD).

29. The viral vector for use according to claim 24 wherein said neurodegenerative disease is Fragile X-associated tremor/ataxia syndrome (FXTAS).

30. A cell transfected with a viral vector according to the invention.

31. The cell according to claim 30 wherein said cell is a neurone and/or an astrocyte, and optionally wherein said neurone is a motor neurone and/or an astrocyte.

32. An isolated nucleic acid molecule encoding an shRNA molecule comprising or consisting of a nucleotide sequence selected from the group consisting of SEQ ID NO 2, 3, 4, 5, 6, 7, 8, 9,10 and 11.

33. The isolated nucleic acid molecule according to claim 32 wherein said nucleic acid molecule comprises or consist of a nucleotide sequence set forth in SEQ ID NO 7.

34. The isolated nucleic acid molecule according to claim 32 wherein said nucleic acid molecule comprises or consist of a nucleotide sequence set forth in SEQ ID NO 10.

35. The isolated nucleic acid molecule according to claims 32 wherein said nucleic acid molecule comprises or consist of a nucleotide sequence set forth in SEQ ID NO 11.

36. A cell penetrating polypeptide comprising or consisting of an amino acid sequence set forth in SEQ ID NO 90.

37. The cell penetrating peptide according to claim 36 wherein said polypeptide is between 13-42 amino acids in length.

38. The cell penetrating peptide according to claim 37 wherein said polypeptide comprises or consist of an amino acid sequence set forth in SEQ ID NO 75.

39. An antagonistic agent comprising a nucleic acid molecule wherein said nucleic acid molecule comprises a nucleotide sequence designed with reference to human Serine/Arginine Rich Splice Factor (SRSF1) and wherein said nucleic acid molecule inhibits expression of SRSF1.

40. The agent according to claim 39 wherein said nucleic acid molecule is a double stranded nucleic acid molecule comprising a sense strand and an antisense strand comprising nucleotide sequences wherein said antisense nucleotide strand is adapted to anneal by complementary base pairing to a nucleic acid molecule encoding human SRSF1.

41. The agent according to claim 40 wherein said double stranded nucleic acid molecule is RNA.

42. The agent according to claim 41 wherein said RNA is siRNA or miRNA.

43. The agent according to claim 39 wherein said nucleic acid molecule is a single stranded nucleotide sequence comprising an antisense nucleotide sequence wherein said antisense nucleotide sequence is adapted to anneal by complementary base pairing to a nucleic acid molecule encoding SRSF1.

44. The agent according to claim 43 wherein said single stranded nucleic acid is DNA and/or RNA, and optionally, said DNA and/or RNA is a therapeutic antisense oligonucleotide such as an antisense oligonucleotide, a splice-switching oligonucleotide, a gapmer or similar.

45. The agent according to claim 44 wherein said DNA is an antisense oligonucleotide.

46. The agent according to any one of claims 39 to 45 wherein said nucleic acid molecule encoding human SRSF1 is set forth in SEQ ID NO: 67.

47. The agent according to claim 46 wherein antagonistic agent comprises a nucleic acid molecule that is at least 15 nucleotides in length.

48. The agent according to claim 47 wherein said antagonistic agent comprises a nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID NO: 67 wherein said nucleic acid molecule is a double stranded inhibitory RNA and is 19-23 nucleotides in length.

49 The agent according to any one of claims 39 to 48 wherein said antagonistic agent comprises a nucleic acid molecule comprising modified nucleotides and/or modified sugars.

50. The agent according to claim 49 wherein said double stranded nucleic acid molecule comprising sense and antisense nucleic acid molecules comprise modified nucleotides.

51. The agent according to claim 50 wherein said modified nucleotides are selected from the group: a 3 '-terminal deoxy- thymine (dT) nucleotide, a 2'-0-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-0-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2' -hydroxly- modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-0- alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising phosphorodithioate (PS2), a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'- phosphate, and a nucleotide comprising a 5 '-phosphate mimic, for example a 5'-vinyl phosphate, a nucleotide comprising a 2'-deoxy-2'-fluro and a 2' methyl sugar base.

52. The agent according to any one of claims 49 to 51 wherein said double stranded nucleic acid molecule comprising sense and antisense nucleic acid molecules comprise modified sugar(s).

53. The agent according to claim 52 wherein said modified sugar is selected from the group: a modified version of the ribosyl moiety, such as -O- modified RNA such as 2'-O-alkyl or 2'-O-(substituted)alkyl e.g. 2'-0-methyl, T-0-(2- cyanoethyl), 2'-0-(2-methoxy)ethyl (2'-MOE), 2'-0-(2-thiomethyl)ethyl, 2'-O-butyryl, -O- propargyl, 2'-O-allyl, 2'-O-(2-amino)propyl, 2'-

O-(2-(dimethylamino)propyl), 2'-O-(2- amino)ethyl, 2'-O-(2-(dimethylamino)ethyl); 2'-deoxy (DNA); 2'-O-(haloalkoxy)methyl, e.g. 2'-0-(2-chloroethoxy)methyl (MCEM), -O- (2,2-dichloroethoxy)methyl (DCEM); 2'-<3-alkoxycarbonyl e.g. T-0-[2- (methoxycarbonyl)ethyl] (MOCE), 2'-O-[2-(N-methylcarbamoyl)ethyl] (MCE), T-0-[2-(N,N- dimethylcarbamoyl)ethyl] (DCME); 2'-halo e.g. 2'-F, FANA (2'-F arabinosyl nucleic acid); carbasugar and azasugar modifications; 3 '-O-alkyl e.g. 3'-0-methyl, 3 '-0-butyryl, V-O- propargyl and their derivatives.

54. The agent according to any one of claims 49 to 53 wherein said antagonistic agent comprises a nucleotide sequence designed with reference to the target nucleic acid sequences selected from the group:

TGGCACTGGTGTCGTGGAGTTTGTA (SEQ ID NO 110);
TGGTGTCGTGGAGTTTGTACGGAAA (SEQ ID NO 111);
TCGTGGAGTTTGTACGGAAAGAAGA (SEQ ID NO 112);
AAGATATGACCTATGCAGTTCGAAA (SEQ ID NO 113);
GAGAAACTGCCTACATCCGGGTTAA (SEQ ID NO 114);
CGGGTTAAAGTTGATGGGCCCAGAA (SEQ ID NO 115);
TGATGGGCCCAGAAGTCCAAGTTAT (SEQ ID NO 116);
CAGAAGTCCAAGTTATGGAAGATCT (SEQ ID NO 117);
GAGAAGCAGAGGATCACCACGCTAT (SEQ ID NO 118); and
CGTCATAGCAGATCTCGCTCTCGTA (SEQ ID NO 119).

55. A pharmaceutical composition comprising an antagonist agent according to any one of claims 39 to 54 and including an excipient or carrier.

56. An antagonistic agent according to any one of claims 39 to 54 for use as a medicament.

57. An antagonistic agent according to any one of claims 39 to 54 for use in the treatment of a neurodegenerative disease.

58. The antagonistic agent according to claim 57 wherein said neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

59. The antagonistic agent according to claim 58 wherein said neurodegenerative disease is sporadic and/or familial amyotrophic lateral sclerosis.

60. The antagonistic agent according to claim 58 or 59 wherein said neurodegenerative disease is ALS not caused by pathological C9ORF72-repeat expansion.

61. The antagonistic agent according to claim 57 wherein said neurodegenerative disease is sporadic frontotemporal dementia (FTD).

62. The antagonistic agent according to claim 57 wherein said neurodegenerative disease is Fragile X-associated tremor/ataxia syndrome (FXTAS).

63. A shRNA molecules comprising a nucleotide sequence, or variant thereof, selected from the group consisting of:
SRSF1-shRNA1 (SEQ ID NO 91):
GCUGAUGUUUACCGAGAUGGC UUCAAGAGA GCCAUCUCGGUAAACAUCAGC;
SRSF1-shRNA2 (SEQ ID NO 92):
GGAGUUUGUACGGAAAGAAGA UUCAAGAGA UCUUCUUUCCGUACAAACUCC;
SRSF1-shRNA3 (SEQ ID NO 93):
GGAAAGAAGAUAUGACCUAUG UUCAAGAGA CAUAGGUCAUAUCUUCUUUCC;
SRSF1-shRNA4 (SEQ ID NO 94):
GAAAGAAGAUAUGACCUAUGC UUCAAGAGA GCAUAGGUCAUAUCUUCUUUC;
SRSF1-shRNA5 (SEQ ID NO 95):
GCCUACAUCCGGGUUAAAGUU UUCAAGAGA AACUUUAACCCGGAUGUAGGC;
SRSF1-shRNA6 (SEQ ID NO 96):
GGGCCCAGAAGUCCAAGUUAU UUCAAGAGA AUAACUUGGACUUCUGGGCCC;
SRSF1-shRNA7 (SEQ ID NO 97):
GGCCCAGAAGUCCAAGUUAUG UUCAAGAGA CAUAACUUGGACUUCUGGGCC;
SRSF1-shRNA8 (SEQ ID NO 98):
GCCCAGAAGUCCAAGUUAUGG UUCAAGAGA CCAUAACUUGGACUUCUGGGC;
SRSF1-shRNA9 (SEQ ID NO 99):
GGAAGAUCUCGAUCUCGAAGC UUCAAGAGA GCUUCGAGAUCGAGAUCUUCC; and
SRSF1-shRNA10 (SEQ ID NO 100):
GCAGAGGAUCACCACGCUAUU UUCAAGAGA AAUAGCGUGGUGAUCCUCUGC.

64. The shRNA molecule according to claim 63 wherein said shRNA molecule comprises or consist of a nucleotide sequence, or variant thereof, set forth in SEQ ID NO 96.

65. The shRNA molecule according to claim 63 wherein said shRNA molecule comprises or consist of a nucleotide sequence, or variant thereof, set forth in SEQ ID NO 99.

66. The shRNA molecule according to claim 63 wherein said shRNA molecule comprises or consist of a nucleotide sequence, or variant thereof, set forth in SEQ ID NO 100.

Intellectual Property Office

Application No:	GB2312083.5	**Examiner:**	Dr Graham Feeney
Claims searched:	In part 1-31	**Date of search:**	5 February 2024

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1-31	US 2019/0194660 A1 (HAUTBERGUE et al.) The whole document, esp. see the claims
A	-	WO 2014/007858 A1 (UNIV IOWA RES FOUND) claim 1
A	-	WO 2017/161273 A1 (THE CHILDREN'S HOSPITAL OF PHILADELPHIA) claim 1

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
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&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

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The following online and other databases have been used in the preparation of this search report

SEARCH-PATENT, SEARCH-NPL, Cas Online

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International Classification:

Subclass	Subgroup	Valid From
C12N	0015/864	01/01/2006
A61K	0031/713	01/01/2006
A61K	0038/16	01/01/2006
A61K	0038/17	01/01/2006
A61P	0025/28	01/01/2006
C07K	0014/47	01/01/2006
C12N	0015/113	01/01/2010