

Summary

Herein are reported novel deoxyribonucleic acids comprising an adenoviral VA RNA and methods using the same. The novel deoxyribonucleic acids according to the current disclosure are useful in the production of recombinant adeno-associated virus particles.

Thus, the present disclosure provides an adenoviral VA RNA nucleic acid. In the adenoviral VA RNA nucleic acid reported herein, the VA RNA coding sequence is operably linked at its 5'-terminus to a variant type 2 polymerase III promoter, or a type 3 polymerase III promoter or variant thereof, such as, e.g., the U6-snRNA promoter, or a polymerase II promoter.

In the adenoviral VA RNA nucleic acid according to the current disclosure the VA RNA coding sequence is operably linked at its 5'-terminus to the U6-snRNA promoter.

In the adenoviral VA RNA nucleic acid according to the current disclosure the VA RNA coding sequence is operably linked at its 5'-terminus to an inducible promoter.

In one preferred example, the adenoviral VA RNA coding sequence has the sequence of SEQ ID NO: 38.

In one aspect, the present disclosure provides an adenoviral VA RNA nucleic acid comprising in 5'- to 3'-direction

- a human U6 RNA promoter, and
- an adenoviral VA RNA coding sequence of SEQ ID NO: 38.

In another aspect, the present disclosure provides an adenoviral VA RNA nucleic acid comprising in 5'- to 3'-direction

- an inducible promoter, and
- an adenoviral VA RNA coding sequence of SEQ ID NO: 38.

5 In one example, the adenoviral VA RNA nucleic acid comprises a precise transcription start site located 3' to the promoter. In one example, the precise transcription start site comprises in 5'- to 3'-direction at least the six 5'-terminal nucleotides of an adenoviral VA RNAI gene comprising the transcription start site (TSS) (to prevent by-passing of the subsequent polymerase III (pol III) terminator) and a functional polymerase III terminator (to prevent transcription from the constitutively active upstream promoter).

In one example, the adenoviral VA RNA nucleic acid comprises a polymerase III terminator at its 3'-terminus.

10 In one example, all elements of the adenoviral VA RNA nucleic acid are arranged in an operably linked form.

In one example, the adenoviral VA RNA nucleic acid is functional.

Without being bound by this theory it is assumed that an improved control of adenoviral VA RNA transcription and thereby AAV particle production can be achieved with the nucleic acid according to the current disclosure.

5 The present disclosure also provides a packaging cell (line) for rAAV particle production, wherein rep/cap genes as well as adenoviral helper genes are (stably) integrated into the genome and wherein the adenoviral VA RNA nucleic acid comprises an adenoviral VA RNA nucleic acid according to the current disclosure.

10 In one preferred example, the rAAV plasmid, comprising the ITRs and the transgene, is also integrated in the packaging cell's genome. Thereby a packaging cell line is turned into a rAAV vector and particle producing cell line. Likewise, in certain examples, the rAAV plasmid/genome is introduced transiently.

15 After recombination, the cells of the producing cell line are genetically uniform and express all genes that are required for replication and packaging of the rAAV in the correct stoichiometry (in contrast thereto, in triple or dual transfection methods some cells may receive suboptimal doses of one or the other plasmids). Thus, without being bound by this theory, a stable rAAV vector/particle packaging or producing cell may result in higher product quality compared to transient packaging or producing cells.

The present disclosure also provides a DNA (molecule) comprising

- 20
- an adenoviral VA RNA nucleic acid according to the current disclosure,
 - a first DNA element,
 - optionally a second DNA element,
 - optionally a third DNA element, and
 - optionally a rep or/and cap open reading frame.

25 In one example

- the first DNA element comprises an E1A open reading frame and an E1B open reading frame; and

- the second DNA element, if present, comprises an E2A open reading frame and an E4 or E4orf6 open reading frame,

or vice versa.

In another aspect, the present disclosure provides a DNA comprising

- 5 - an adenoviral VA RNA nucleic acid according to the present disclosure, and
- a DNA element comprising
 - an E1A open reading frame and an E1B open reading frame; or
 - an E2A open reading frame and an E4 or E4orf6 open reading
 - 10 frame; or
 - a rep open reading frame and a cap open reading frame.

The present disclosure also provides a mammalian or insect cell comprising an adenoviral VA RNA nucleic acid or a DNA (element) according to the current disclosure.

- 15 In another aspect, the present disclosure provides a mammalian or insect cell comprising an adenoviral VA RNA nucleic acid according to the present disclosure or a DNA according to the present disclosure.

The present disclosure also provides a method for producing a recombinant adeno-associated virus (rAAV) vector or particle comprising the following steps:

- 20 - cultivating/propagating a cell according to the current disclosure (under conditions suitable for cell division), and
- recovering the rAAV vector or particle from the cells or the cultivation medium.

The present disclosure also provides an adenoviral VA RNA nucleic acid or a DNA (molecule) according to the current disclosure for the production of recombinant adeno-associated virus vectors or particles.

5 The present disclosure also provides an adenoviral VA RNA nucleic acid, wherein the wild-type type 2 polymerase III promoter has been inactivated/deleted/removed, and the U6-snRNA promoter has been added. In one example, further a precise transcription start site has been added.

10 The present disclosure also provides a method of generating/for producing a recombinant adeno-associated virus (rAAV) vector or particle, wherein the method comprises:

- generating/providing a mammalian, in suspension growing cell, which comprises either stably integrated into its genome or transiently present
- a transgene expression cassette interspaced between two AAV ITRs;
- 15 - open reading frames encoding adenoviral E1A, E1B, E2A, E4 or E4orf6 proteins and an adenoviral VA RNA nucleic acid according to the current disclosure;

- open reading frames encoding adeno-associated Rep/Cap proteins;
- propagating/cultivating the mammalian cell (under conditions to allow cell division); and
- isolating the rAAV vector or particle from the cell or the cultivation medium and thereby producing the rAAV vector or particle.

In another aspect, the present disclosure provides a method for producing recombinant adeno-associated virus particles comprising:

- providing a mammalian, in suspension growing cell, which comprises
 - a transgene expression cassette interspaced between two AAV ITRs;
 - open reading frames encoding adenoviral E1A, E1B, E2A, E4 or E4orf6 proteins;
 - an adenoviral VA RNA nucleic acid according to the present disclosure;
 - open reading frames encoding adeno-associated Rep/Cap proteins;
- cultivating the mammalian cell; and
- isolating the rAAV particles from the cell or the cultivation medium and thereby producing rAAV particles.

Detailed Description of Embodiments of the Invention

Herein are reported novel nucleic acids and DNA elements as well as methods using the same. The nucleic acids according to the current invention are useful in the recombinant production of AAV particles. The current invention uses a deliberate arrangement of promoter and coding sequence to provide a novel adenoviral VA RNA nucleic acid.

DEFINITIONS

Useful methods and techniques for carrying out the current invention are described in e.g. Ausubel, F.M. (ed.), Current Protocols in Molecular Biology, Volumes I to III (1997); Glover, N.D., and Hames, B.D., ed., DNA Cloning: A Practical Approach, Volumes I and II (1985), Oxford University Press; Freshney, R.I. (ed.), Animal Cell Culture – a practical approach, IRL Press Limited (1986); Watson, J.D., et al., Recombinant DNA, Second Edition, CHSL Press (1992); Winnacker, E.L., From Genes to Clones; N.Y., VCH Publishers (1987); Celis, J., ed., Cell Biology, Second Edition, Academic Press (1998); Freshney, R.I., Culture of Animal Cells: A Manual of Basic Technique, second edition, Alan R. Liss, Inc., N.Y. (1987).

The use of recombinant DNA technology enables the generation of derivatives of a nucleic acid. Such derivatives can, for example, be modified in individual or several nucleotide positions by substitution, alteration, exchange, deletion or insertion. The modification or derivatization can, for example, be carried out by means of site directed mutagenesis. Such modifications can easily be carried out by a person skilled in the art (see e.g. Sambrook, J., et al., Molecular Cloning: A laboratory manual (1999) Cold Spring Harbor Laboratory Press, New York, USA; Hames, B.D., and Higgins, S.G., Nucleic acid hybridization – a practical approach (1985) IRL Press, Oxford, England).

Deoxyribonucleic acids comprise a coding and a non-coding strand. The terms “5’-” and “3’-” when used herein refer to the position on the coding strand.