WO 2018/150271 reported a mammalian cell comprising at least four distinct recombination target sites (RTS), an adenoviral (Ad) gene comprising E1A, E1B or a combination thereof, and a promoter operatively linked to the Ad gene, wherein the RTS, the Ad gene, and the promoter are chromosomally-integrated; methods for using the cell for generating a recombinant adeno-associated vims (rAAV) producer host cell; and methods for using the AAV producer host cell to produce, package and purify rAAV.

Mingqi, X., et al. reported about mammalian designer cells - engineering principles and biomedical applications (Biotechnol. J. 10 (2015) 1005-1018.

Thus, there is a need for functional genomics tools, with which the number of transgenic DNA segments that can be selectively addressed in a genomic sequence is increased.

Summary

Herein are reported novel deoxyribonucleic acids and methods using the same. The novel deoxyribonucleic acids according to the current disclosure are useful in the simultaneous activation of the expression of at least two open reading frames/genes by site-specific recombinase technology. The current disclosure uses a deliberate inactive arrangement of promoters and open reading frames/gene elements on the coding strand (the (+) strand, the positively oriented strand) and on the template strand (the (-) strand, the negatively oriented strand) of deoxyribonucleic (DNA) molecules, which require for transcriptional activation, i.e. operable linkage of promoter and coding sequence allowing transcription of said coding sequence, inversion by the interaction with a site-specific recombinase.

The present disclosure provides a recombinase-activatable 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 at least one of them, in one preferred example at least two of them, is comprised in a deoxyribonucleic acid according to the current disclosure and can thereby be transcriptionally activated by the interaction with a site-specific recombinase. In certain examples, the transcriptional activation of one or more adenoviral helper genes is accomplished by recombinase-mediated open reading frame/gene inversion (RMCI). For example, after its activation the adenoviral helper protein E1A activates the transcription of the rep gene from the autologous P5 promoter, which in turn activates transcription of the cap gene. In certain examples, rep/cap gene transcription is activated using

recombinase-mediated open reading frame/gene inversion in a deoxyribonucleic acid according to the current disclosure, in cells in which the adenoviral E1A protein is constitutively expressed, as for instance in HEK cells, or a heterologous promoter is used to drive rep and/or cap gene transcription. In a certain example, the recombinase is Cre-recombinase form bacteriophage P1.

Cre-recombinase expression is, in certain examples, induced by transient transfection of small amounts of a Cre-recombinase encoding nucleic acid. It has been found that efficient recombination can be accomplished with as little as 10 % of the amount of plasmid DNA that is usually used for transient virus production. Even lower amounts of nucleic acid are sufficient if Cre-recombinase encoding mRNA is used. In certain examples, a Cre-recombinase encoding nucleic acid is integrated into the packaging cell line's genome and operably linked to an inducible promoter, such as, e.g., a Tet-inducible promoter. In one preferred example, the rAAV genome, comprising the ITRs and the transgene, is also integrated in the packaging cell line's genome. Thereby a packaging cell line is turned into a rAAV vector and particle producing cell line. Likewise, in certain examples, the rAAV genome is introduced transiently.

After recombination, the cells of the producing cell line are genetically uniform and express all genes that are required for rAAV replication and packaging 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/genes). Thus, without being bound by this theory, a stable rAAV vector/particle packaging or producing cell line may result in higher product quality compared to transient packaging or producing cells. In addition, induction of rAAV vector or particle production by transfection with a Cre-recombinase encoding nucleic acid instead of a helper virus provides for improved safety of the produced rAAV vector/particle.

The present disclosure further provides a novel adenoviral VA RNA gene. The adenoviral VA RNA gene according to the current disclosure enables Cre-recombinase mediated gene activation by inversion. In the adenoviral VA RNA according to the current disclosure, the adenoviral VA RNA gene can be driven by any promoter with a precise transcription start site together with a LoxP site introduced into the non-coding, i.e. regulatory, elements of the adenoviral VA RNA.

The present disclosure further provides the novel LoxP site (spacer sequence) AGTTTATA (SEQ ID NO: 01 (forward orientation); SEQ ID NO: 02 (reverse

orientation)). This spacer sequence is termed Lx herein. It can be combined with any known left and right repeat sequences.

In certain examples, the Lx spacer sequence is combined with a mutated left inverted repeat and a wild-type right inverted repeat. This Cre-recombinase recognition sequence is denoted as Lx-LE and has in forward orientation the sequence of SEQ ID NO: 03 and in reverse orientation the sequence of SEQ ID NO: 04.

In certain examples, the Lx spacer sequence is combined with a mutated right inverted repeat and a wild-type left inverted repeat. This Cre-recombinase recognition sequence is denoted as Lx-RE and has in forward orientation the sequence of SEQ ID NO: 05 and in reverse orientation the sequence of SEQ ID NO: 06.

The technical principle underlying the current disclosure is transcriptional activation of open reading frames or genes by combining DNA-inversion with concomitant operable-linking to a regulatory element, such as, e.g., a promoter.

The present disclosure provides a double stranded DNA element comprising a (positively oriented) coding strand and a (negatively oriented) template strand,

characterized in that

the coding strand comprises in 5'- to 3'-orientation, i.e. in the following order

- a first promoter,
- a first recombinase recognition sequence comprising a mutation in one of the inverted repeats, i.e. either in the left inverted repeat or in the right inverted repeat, and the other inverted repeat is a not-mutated/wild-type inverted repeat,
- a second promoter that is inverted (in sequence) with respect to the coding strand (direction),
- a first polyadenylation signal and/or transcription termination element, which is inverted (in sequence) with respect to the coding strand (direction),

- a first open reading frame that is inverted (in sequence) with respect to the coding strand (direction) and that is operably linked to the first polyadenylation signal and/or transcription termination element,

- a second recombinase recognition sequence, which comprises a mutation in the respective other inverted repeat as the first recombinase recognition sequence, and which is in inverted/reciprocal orientation with respect to the first recombinase recognition sequence,

- a second open reading frame,

- a second polyadenylation signal and/or transcription termination element, which is operably linked to the second open reading frame.

In one aspect, the present disclosure provides a double stranded DNA element comprising a coding strand and a template strand,

characterized in that

the coding strand comprises in 5'- to 3'-orientation in the following order

- a first promoter,

- a first recombinase recognition sequence comprising a mutation in the left inverted repeat,

- a second promoter that is inverted with respect to the coding strand,

- a first polyadenylation signal sequence and/or transcription termination element that is/are inverted with respect to the coding strand,

- a first open reading frame that is inverted with respect to the coding strand and that is operably linked to the first polyadenylation signal sequence and/or transcription termination element,

- a second recombinase recognition sequence comprising a mutation in the right inverted repeat and in inverted orientation with respect to the first recombinase recognition sequence,

- a second open reading frame, and

- a second polyadenylation signal sequence and/or transcription termination element operably linked to the second open reading frame.

The present disclosure provides a double stranded DNA element comprising in 5'- to 3'-direction, i.e. in the following order

- a first promoter in 5'- to 3'-orientation / positive orientation,

- a first recombinase recognition sequence comprising a mutation in one of the inverted repeats, i.e. either in the left inverted repeat or in the right inverted repeat,

- a second promoter in 3'- to 5'-orientation / negative orientation,

- a first polyadenylation signal and/or transcription termination element in 3'- to 5'-orientation / negative orientation,

- a first open reading frame in 3'- to 5'-orientation / negative orientation and operably operably linked to the first polyadenylation signal and/or transcription termination element,

- a second recombinase recognition sequence, which comprises a mutation in the respective other inverted repeat as the first recombinase recognition sequence, and which is in reciprocal/inverted orientation with respect to the first recombinase recognition sequence,

- a second open reading frame in 5'- to 3'-orientation / positive orientation,

- a second polyadenylation signal and/or transcription termination element, which is operably linked to the second open reading frame.

In certain examples, incubation of the double stranded DNA element with a recombinase functional with said first and second recombinase recognition sequence results

- in the inversion of the sequence located between the first and the second recombinase recognition sequence, whereafter the first promoter is operably linked to the first open reading frame and the second promoter is operably linked to the second open reading frame, and

- in the generation of a (third) recombinase recognition sequence between the first promoter and the first open reading frame or between the second promoter and the second open reading frame following recombinase-mediated inversion of the DNA sequence between said first and second recombinase recognition sequence, which ((third) recombinase recognition sequence) is no-longer functional with said recombinase.

The present disclosure provides a double stranded adenoviral VA RNA element comprising in 5'- to 3'-direction, i.e. in the following order

- a promoter in 5'- to 3'-orientation / positive orientation,

- a first recombinase recognition sequence comprising a mutation in one of the inverted repeats, i.e. either in the left inverted repeat or in the right inverted repeat,

- an adenoviral VA RNA gene in 3'- to 5'-orientation / negative orientation,

- a second recombinase recognition sequence, which comprises a mutation in the respective other inverted repeat as the first recombinase recognition sequence, and which is in reciprocal/inverted orientation with respect to the first recombinase recognition sequence.

In certain examples, incubation of the double stranded VA RNA element with a recombinase functional with said first and second recombinase recognition sequence results

- in the inversion of the sequence between the first and the second recombinase recognition sequence, whereafter the promoter is operably linked to the VA RNA gene, and

- in the generation of a (third) recombinase recognition sequence between the promoter and the VA RNA gene or downstream of the VA RNA gene following recombinase-mediated inversion of the DNA sequence between said first and second recombinase recognition sequence, which ((third) recombinase recognition sequence) is no-longer functional with said recombinase.

The present disclosure provides a (double stranded) DNA (molecule) comprising

- a first double stranded DNA element according to the disclosure,

- a second double stranded DNA element according to the disclosure,

- optionally a third double stranded DNA element according to the disclosure or an adenoviral VA RNA element according to the disclosure, and

- a rep or/and cap open reading frame (element).

In certain examples,

1)

- in the first double stranded DNA element the first open reading frame is the E1A open reading frame and the second open reading frame is the E1B open reading frame, or vice versa; and

- in the second double stranded DNA element the first open reading frame is the E2A open reading frame and the second open reading frame is the E4 open reading frame or the E4orf6 (open reading frame), or vice versa,

or

2)

- in the first double stranded DNA element the first open reading frame is the E2A open reading frame and the second open reading frame is the E4 open reading frame or the E4orf6 (open reading frame), or vice versa; and

- in the second double stranded DNA element the first open reading frame is the E1A open reading frame and the second open reading frame is the E1B open reading frame, or vice versa.

The present disclosure provides a mammalian or insect cell comprising at least one double stranded DNA element or molecule according to the current disclosure or a (sequence) inverted form thereof.

In another aspect, the present disclosure provides a mammalian cell comprising

- one or more double stranded DNA elements according to the present disclosure, or
- at least one double stranded DNA element according to the present disclosure, or
- one double stranded DNA molecule according to the present disclosure and one double stranded DNA molecule according to the present disclosure, or
- at least one double stranded DNA molecule according to the present disclosure, or
- one or more double stranded DNAs according to the present disclosure.

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

- cultivating/propagating a cell according to the current disclosure (under conditions suitable for cell division),

- activating rAAV vector or particle production by recombinase mediated open reading frame inversion according to the disclosure (by introducing a recombinase as protein or as mRNA or as DNA in the cell according to the disclosure, whereby the recombinase is functional with the recombinase recognition sequences in the DNA element or molecule according to the disclosure),

- optionally cultivating the rAAV vector or particle production activated cell obtained in the previous step (under conditions suitable for rAAV vector or particle production),

- recovering the rAAV vector or particle from the cells or/and the cultivation medium.

The present disclosure provides a (double stranded) DNA (molecule) (for the production of recombinant adeno-associated virus vectors or particles) comprising

a) an E1A open reading frame and an E1B open reading frame; and

b) an E2A open reading frame and an E4 or E4orf6 open reading frame;

characterized in that the first and second open reading frames of a) or b) are comprised/contained in a double stranded DNA element comprising a (positively oriented) coding strand and a (negatively oriented) template strand,

wherein the coding strand comprises in 5'- to 3'-orientation, i.e. in the following order

- a first promoter (in positive orientation),
- a first recombinase recognition sequence comprising a mutation in one of the inverted repeats,
- a second promoter that is inverted (in sequence) with respect to the coding strand (direction) (i.e. is in inverted/negative orientation),
- optionally a first polyadenylation signal and/or transcription termination element that is inverted (in sequence) with respect to the coding strand (direction) (i.e. is in inverted/negative orientation) and that is operably linked to the first open reading frame,
- the first open reading frame (of a) or b)) that is inverted (in sequence) with respect to the coding strand direction (i.e. is in inverted/negative orientation),
- a second recombinase recognition sequence comprising a mutation in the respective other inverted repeat and being in reciprocal/inverted orientation with respect to the first recombinase recognition sequence,
- the second open reading frame of a) if the first open reading frame is of a) or the second open reading frame of b) if the first open reading frame is of b) (in positive orientation),
- optionally a second polyadenylation signal and/or transcription termination element (in positive orientation and operably linked to the second open reading frame).

In another aspect, the present disclosure provides a double stranded DNA molecule comprising

a) the E1A open reading frame and the E1B open reading frame; and/or

b) the E2A open reading frame and the E4orf6 open reading frame;

characterized in that

the first and second open reading frames of a) or/and b) are contained in a double stranded DNA element comprising a coding strand and a template strand,

wherein the coding strand comprises in 5'- to 3'-orientation in the following order

- a first promoter,

- a first recombinase recognition sequence comprising a mutation in the right inverted repeat,

- a second promoter that is inverted with respect to the coding strand,

- the first open reading frame of a) or b) that is inverted with respect to the coding strand,

- a second recombinase recognition sequence comprising a mutation in the left inverted repeat and in inverted orientation to the first recombinase recognition sequence, and

- the second open reading frame of a) or b).

The present disclosure provides a (double stranded) DNA (molecule) (for the production of recombinant adeno-associated virus vectors or particles) comprising

a) an E1A open reading frame and an E1B open reading frame; and

b) an E2A open reading frame and an E4 or E4orf6 open reading frame;

characterized in that the first and the second open reading frames of a) and the first and the second open reading frames b) are each contained in a double stranded DNA element (i.e. the DNA molecule comprises two of said DNA elements) each comprising a (positively oriented) coding strand and a (negatively oriented) template strand,

wherein the coding strand comprises in 5'- to 3'-orientation, i.e. in the following order

- a first promoter (in positive orientation),
- a first recombinase recognition sequence comprising a mutation in one of the inverted repeats,
- a second promoter that is inverted (in sequence) with respect to the coding strand (direction) (i.e. is in inverted/negative orientation),
- optionally a first polyadenylation signal and/or transcription termination element that is inverted (in sequence) with respect to the coding strand (direction) (i.e. is in inverted/negative orientation) and that is operably linked to the first open reading frame,
- the first open reading frame (of a) or b)) that is inverted (in sequence) with respect to the coding strand direction (i.e. is in inverted/negative orientation),
- a second recombinase recognition sequence comprising a mutation in the respective other inverted repeat and being in reciprocal/inverted orientation with respect to the first recombinase recognition sequence,
- the second open reading frame of a) if the first open reading frame is of a) or the second open reading frame of b) if the first open reading frame is of b) (in positive orientation),
- optionally a second polyadenylation signal and/or transcription termination element (in positive orientation and operably linked to the second open reading frame).

Thus, the present disclosure provides a (double stranded) DNA (molecule) (for the production of recombinant adeno-associated virus vectors or particles)

comprising (at least one) a double stranded DNA element comprising a (positively oriented) coding strand and a (negatively oriented) template strand,

wherein the coding strand comprises in 5'- to 3'-orientation, i.e. in the following order

- a first promoter, in one preferred example the adeno-associated viral promoter P5 or a functional fragment thereof or a variant thereof,
- a first recombinase recognition sequence comprising a mutation in one of the inverted repeats,
- the rep and cap open reading frames including further promoters for the expression of the Rep and Cap proteins, which are inverted (in sequence) with respect to the coding strand (direction) (i.e. in inverted orientation),
- a second recombinase recognition sequence comprising a mutation in the respective other inverted repeat and being in reciprocal/inverted orientation to the first recombinase recognition sequence,
- a polyadenylation signal, in one preferred example the autologous polyadenylation signal of the rep and cap open reading frames.

In another aspect, the present disclosure provides a double stranded DNA element comprising a coding strand and a template strand, wherein the coding strand comprises in 5'- to 3'-orientation in the following order

- a first promoter,
- a first recombinase recognition sequence comprising a mutation in the left inverted repeat,
- a Rep/Cap open reading frames including further promoters for the expression of the Rep and Cap proteins, which is inverted with respect to the coding strand,

- a second recombinase recognition sequence comprising a mutation in the right inverted repeat and in inverted orientation with respect to the first recombinase recognition sequence, and

- a polyadenylation signal sequence.

In certain examples, incubation of the (double stranded) DNA (molecule) with a recombinase functional with said first and second recombinase recognition sequence results

- in the inversion of the sequence between the first and the second recombinase recognition sequence, whereafter the first promoter is operably linked to the rep and cap open reading frames, and

- in the generation of a (third) recombinase recognition sequence between the first promoter and the rep and cap open reading frames or between the rep and cap open reading frames and the polyadenylation signal following recombinase-mediated inversion of the DNA sequence between said first and second recombinase recognition sequence, which the first and the second open reading frames is no-longer functional with said recombinase.

The present disclosure further provides a (double stranded) DNA (molecule) (for the production of recombinant adeno-associated virus vectors or

particles) comprising a double stranded DNA element comprising a (positively oriented) coding strand and a (negatively oriented) template strand,

wherein the coding strand comprises in 5'- to 3'-orientation, i.e. in the following order

- a first promoter, in one preferred example the adeno-associated viral promoter P5 or a functional fragment thereof or a variant thereof,

- a first recombinase recognition sequence comprising a mutation in one of the inverted repeats,

- a second promoter that is inverted with respect to the coding strand (in inverted orientation), in one preferred example the adeno-associated viral promoter P19 or a functional fragment thereof or a variant thereof,

- optionally a first polyadenylation signal and/or transcription termination element that is inverted (in sequence) with respect to the coding strand (direction) (i.e. is in inverted/negative orientation) and that is operably linked to the Rep78 or Rep68 coding sequence,

- a coding sequence, which encodes either exclusively the Rep78 protein or exclusively the Rep68 protein, but not both,
  (i) optionally the internal P40 promoter is inactivated, and/or
  (ii) the start codon of Rep52/40 is mutated into a non-start codon, and/or
  (iii) splice donor and acceptor sites are removed,
  and which is inverted with respect to the coding strand (in inverted orientation),

- a second recombinase recognition sequence, which comprises a mutation in the respective other inverted repeat as the first recombinase recognition sequence, and which is in reciprocal/inverted orientation with respect to the first recombinase recognition sequence,

- the Rep52/Rep40 and Cap open reading frames including a common polyadenylation signal sequence, i.e. a polyadenylation signal operably linked to said open reading frames.

The present disclosure further provides a (double stranded) DNA (molecule) (for the production of recombinant adeno-associated virus vectors or particles) comprising a double stranded DNA element comprising a (positively oriented) coding strand and a (negatively oriented) template strand,

wherein the coding strand comprises in 5'- to 3'-orientation, i.e. in the following order

- a first promoter, in one preferred example the adeno-associated viral promoter P5 or a functional fragment thereof or a variant thereof,
- a first recombinase recognition sequence comprising a mutation in one of the inverted repeats,
- a second promoter that is inverted with respect to the coding strand (in inverted orientation), in one preferred example the adeno-associated viral promoter P19 or a functional fragment thereof or a variant thereof,
- optionally a first polyadenylation signal and/or transcription termination element that is inverted (in sequence) with respect to the coding strand (direction) (i.e. is in inverted/negative orientation) and that is operably linked to the Rep78 or Rep68 coding sequence,
- a coding sequence, which encodes either exclusively the Rep78 protein or exclusively the Rep68 protein, but not both,
  (i) optionally the internal P40 promoter is inactivated, and/or
  (ii) the start codon of the Rep52/40 open reading frame is mutated into a non-start codon, and
  (iii) splice donor and acceptor sites are removed,
  and which is inverted with respect to the coding strand (in inverted orientation),
- a second recombinase recognition sequence, which comprises a mutation in the respective other inverted repeat as the first recombinase recognition sequence, and which is in reciprocal/inverted orientation with respect to the first recombinase recognition sequence,
- the Rep52 open reading frame, optionally with splice donor and acceptor sites removed, or the Rep 40 open reading frame including a

polyadenylation signal sequence, i.e. a polyadenylation signal operably linked to said open reading frame,

- optionally a third promoter, a cap open reading frame and a polyadenylation and/or terminator sequence, wherein all are operably linked.

In another aspect, the present disclosure provides a double stranded DNA element comprising a coding strand and a template strand,

a) wherein the coding strand comprises in 5'- to 3'-orientation in the following order

- a first promoter,

- a first recombinase recognition sequence comprising a mutation in the left inverted repeat,

- a second promoter that is inverted with respect to the coding strand,

- a first polyadenylation signal sequence and/or transcription termination element that is inverted with respect to the coding strand,

- a coding sequence,
which encodes either exclusively the Rep78 protein or exclusively the Rep68 protein, but not both, wherein
(i) optionally the internal P40 promoter is inactivated, and/or
(ii) the start codon of Rep52/40 is mutated into a non-start codon, and/or
(iii) splice donor and acceptor sites are removed,
which is inverted with respect to the coding strand, and
which is operably linked to the a first polyadenylation signal sequence and/or transcription termination element,

- a second recombinase recognition sequence, which comprises a mutation in the right inverted repeat, and which is in inverted orientation with respect to the first recombinase recognition sequence, and

- a Rep52/Rep40 and Cap open reading frames including a polyadenylation signal operably linked to said open reading frames,

or

b) wherein the coding strand comprises in 5'- to 3'-orientation in the following order

- a first promoter,

- a first recombinase recognition sequence comprising a mutation in the left inverted repeat,

- a second promoter that is inverted with respect to the coding strand,

- a first polyadenylation signal sequence and/or transcription termination element in that is inverted with respect to the coding strand,

- a coding sequence,
which encodes either exclusively the Rep78 protein or exclusively the Rep68 protein, but not both, wherein
(i) optionally the internal promoter is inactivated, and/or
(ii) the start codon of the Rep52/40 open reading frame is mutated into a non-start codon, and
(iii) splice donor and acceptor sites are removed,
which is inverted with respect to the coding strand, and
which is operably linked to the first polyadenylation signal sequence and/or transcription termination element,

- a second recombinase recognition sequence, which comprises a mutation in the right inverted repeat, and which is in inverted orientation with respect to the first recombinase recognition sequence, and

- the Rep52 open reading frame, optionally with splice donor and acceptor sites removed, or the Rep40 open reading frame including a polyadenylation signal operably linked to said open reading frame.

The present disclosure provides an adenoviral VA RNA gene operably linked to a functional promoter, wherein a precise transcription start site has been added and a Cre-recombinase recognition sequence has been engineered into/within the adenoviral VA RNA gene.

The present disclosure provides an isolated (mammalian or insect) cell comprising at least one of the DNA element or the DNA (molecule) or the adenoviral VA RNA of the current disclosure in original or (recombinase) inverted form.

The present disclosure provides a method of generating/for producing a recombinant adeno-associated virus (rAAV) vector or particle, the method 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 and adenoviral VA RNA;

  - open reading frames encoding adeno-associated Rep/Cap proteins;

  - one or more different pairs of non-compatible recombinase recognition sequences;

    wherein individually or in combination one or more from the group consisting of the E1A open reading frame, the E1B open reading frame, the E2A open reading frame, the E4 open reading frame, the E4 open reading frame 6, the Rep78 open reading frame, the Rep68 open reading frame, the Rep52 open reading frame, the Rep40 open reading frame, the Rep/Cap open reading frames and the adenoviral VA RNA gene, is/are each placed without operably linked promoter but including operably linked polyadenylation and/or transcription termination signal between a pair of said non-compatible recombinase recognition sequences, wherein one

recombinase recognition sequence comprises a mutation in the left inverted repeat and one recombinase recognition sequence comprises a mutation in the right inverted repeat, with a promoter located upstream of the first recombinase recognition sequence, and the open reading frame being in reverse orientation with respect to the promoter located upstream therefrom;

wherein the recombinase recognition sequences are organized to allow generation of a recombinase-dependent change that is detectable (e.g. by rAAV vector or particle production), in certain examples the one or more recombinase recognition sequences are Cre-recombinase recognition sites (i.e. the recombinase recognition sequences are in reciprocal/inverted orientation with respect to each other and action of the recombinase results in the inversion of the sequence(s) between the recombinase recognition sequences and the concomitant operable linking to the upstream located promoter to the inverted sequence), in certain examples the one or more recombinase recognition sequences are Flp-recognition sites (i.e. the recombinase recognition sequences are in reciprocal/inverted orientation with respect to each other and action of the recombinase results in the inversion of the sequence(s) between the recombinase recognition sequences and the concomitant operable linking to the upstream located promoter to the inverted sequence);

- inducing expression of the recombinase in said mammalian cell either by transfecting said cell with a recombinase expression plasmid or recombinase mRNA or by activating a conditional recombinase expression within said mammalian cell, whereby the expression of the recombinase results in a recombinase-mediated cassette inversion resulting in rAAV vector or particle production, and wherein the recombinase-mediated cassette inversion is the inversion of the sequence that is flanked by the recombinase recognition sequences;

- isolating the rAAV vector or particle from the cell or/and the cultivation medium and thereby producing the rAAV vector or particle.

The present disclosure provides a method of obtaining site-specific replacement of a DNA of interest in a mammalian cell, comprising:

a) providing a mammalian cell comprising a DNA element according to the current disclosure;

b) introducing into the cell or activating in the cell a recombinase functional with the recombinase recognition sequences of said DNA element of a);

wherein the recombinase catalyzes the inversion of the sequence between the recombinase recognition sequences and thereby a site-specific replacement of a DNA of interest in a mammalian cell is obtained.

In certain examples, the first recombinase recognition sequence comprises a mutation in the left inverted repeat and the second recombinase recognition sequence comprises a mutation in the right inverted repeat. This arrangement results after recombinase-mediated inversion that the upstream, i.e. 5'-located, recombinase recognition sequence comprises a mutation in both inverted repeats and is thereby non-functional, i.e. cannot be recognized by the respective recombinase. The downstream, i.e. 3'-located, recombinase recognition sequence is wild-type with respect to both inverted repeats and is thereby functional, i.e. can be recognized by the respective recombinase.

In certain examples, the first recombinase recognition sequence comprises a mutation in the right inverted repeat and the second recombinase recognition sequence comprises a mutation in the left inverted repeat. This arrangement results after recombinase-mediated inversion that the downstream, i.e. 3'-located, recombinase recognition sequence comprises a mutation in both inverted repeats and is thereby non-functional, i.e. cannot be recognized by the respective recombinase. The upstream, i.e. 5'-located, recombinase recognition sequence is wild-type with respect to both inverted repeats and is thereby functional, i.e. can be recognized by the respective recombinase.

In certain examples, the first promoter is in positive orientation and/or the second open reading frame is in positive orientation.

In another aspect, the present disclosure provides a double stranded DNA molecule comprising two or more double stranded DNA elements or molecules as described herein.

Detailed Description of Embodiments of the Invention

Herein are reported novel DNA constructs and methods using the same. The novel DNA constructs according to the current invention are useful in the simultaneous transcriptional activation of at least two open reading frames or genes using site-specific, recombinase-mediated cassette inversion (RMCI). The current invention uses a deliberate non-productive arrangement of promoters and open reading frames on coding and template strands of double-stranded DNA molecules, which are