result in contaminants (*e.g.*, nucleic acid contaminants) from the cells used to produce the vectors (*e.g.*, Sf9 cells) that are inconvenient or costly to remove and which may have undesirable side effects if included in ceDNA therapeutic formulation. Accordingly, there is need in the field for a technology that allows for the generation of recombinant vectors to be used in methods of controlling gene expression with minimal off-target effects such as those introduced by such contaminants or other artifacts of the purification method. It would also be advantageous that the technology is suitable for and supports large-scale manufacturing of the recombinant vectors. The methods provided herein addresses these unmet needs.

SUMMARY

[0009a] In one aspect, provided herein is a method of producing a closed-ended DNA (ceDNA) vector, the method comprising:

(a) contacting a double-stranded DNA construct having a sense strand and an antisense strand with at least a first restriction endonuclease and at least a second restriction endonuclease, wherein the double-stranded DNA construct comprises:

a transgene expression cassette,

a first non-palindromic restriction endonuclease recognition site and a corresponding first cleavage site upstream of the transgene expression cassette, and

a second non-palindromic restriction endonuclease recognition site and a corresponding second cleavage site downstream of the transgene expression cassette;

wherein the first restriction endonuclease is capable of cleaving the double-stranded DNA construct at the first cleavage site, and wherein the second restriction endonuclease is capable of cleaving the double-stranded DNA construct at the second cleavage site,

and wherein contacting the double-stranded DNA construct with the first restriction endonuclease and the second restriction endonuclease releases an insert having a first end comprising a first single-stranded overhang and a second end comprising a second single-stranded overhang;

(b) ligating the first end comprising the first single-stranded overhang to a first oligonucleotide comprising one or more hairpin structures; and

(c) ligating the second end comprising the second single-stranded overhang to a second oligonucleotide comprising one or more hairpin structures;

thereby producing the ceDNA vector

[0009b] In another aspect, provided herein is a pharmaceutical composition comprising the ceDNA vector as described herein and at least one pharmaceutically acceptable excipient.

[0009c] In another aspect, provided herein is a lipid nanoparticle composition comprising the ceDNA vector as described herein.

[0009d] In another aspect, provided herein is an isolated host cell comprising the ceDNA vector as described herein.

[0009e] In another aspect, provided herein is a transgenic animal comprising the ceDNA vector as described herein.

[0009f] In yet another aspect, provided herein is the ceDNA vector as described herein, or the pharmaceutical composition as described herein, or the lipid nanoparticle composition as described herein for use in the treatment of a disorder, disease, or condition in a subject or delivery of a therapeutic protein to a subject.

[0009g] In yet another aspect, provided herein is a kit for preparing a closed-ended DNA (ceDNA) vector comprising a transgene, the kit comprising:

the DNA vector as described herein;

at least one restriction endonuclease capable of cleaving the DNA vector at the multiple cloning site to allow the multiple cloning site to receive a transgene;

at least one restriction endonuclease capable of cleaving at the first and second cleavage sites;

a ligase; and

instructions for use.

[0009h] In yet another aspect, provided herein is a method of producing a closed-ended DNA (ceDNA) vector, the method comprising:

(a) producing a double-stranded DNA construct using the method as described herein;

(b) performing the method as described herein to produce a ceDNA vector from the double-stranded DNA construct.

[0009i] In yet another aspect, provided herein is a A method of preparing a closed-ended DNA (ceDNA) vector, the method comprising:

(a) contacting a double-stranded DNA construct with at least one restriction endonuclease, wherein the restriction endonuclease is a Type IIs restriction endonuclease, wherein the construct comprises:

a transgene expression cassette;

a first non-palindromic restriction endonuclease recognition site and a corresponding first cleavage site upstream of the transgene expression cassette, and

a second non-palindromic restriction endonuclease recognition site and a corresponding second cleavage site downstream of the transgene expression cassette;

the Type IIs one restriction endonuclease is capable of cleaving the construct at the first and second cleavage sites to release an insert having single-stranded overhangs at the 5’

and 3' ends of the insert that are compatible with the overhangs of a first inverted terminal repeat (ITR) oligonucleotide and a second inverted terminal repeat (ITR) oligonucleotide; and

(b) ligating the 5' and 3' ends of the insert to the first inverted terminal repeat (ITR) oligonucleotide and the second ITR oligonucleotide to form the ceDNA vector.

[0010] Conventional methods for production of viral and virally-derived DNA typically use eukaryotic cells, *e.g.*, mammalian or insect cells. One commonly used insect cell line is Sf9. However, not only do these cells both contain enzymes and other proteins which may have a deleterious effect on the DNA to be replicated, but the process of purifying the desired DNA from cell lysates introduces cellular nucleic acids whose presence can make purification of the desired DNA product more difficult. Furthermore, such impurities or contaminants can have a range of deleterious and/or unwanted effects in the subject to which the desired DNA is administered. Additionally, such traditional cell-based production methods can have issues with respect to the quantity of DNA vector product produced, and it is not uncommon for significant engineering of the cell line itself or the production technology to be required to produce desirable yields.

[0011] The technical solution to these vector production challenges, as provided herein, relates to a *cell-free* synthetic production method that can readily produce closed circle hairpin loop-containing DNA vectors such as, but not limited to, close-ended DNA vectors (ceDNA vectors) in *higher purities* than by conventional means using eukaryotic cells (*see, e.g.*, **FIG. 13**).

[0012] Accordingly, provided herein is a method of producing a closed-ended DNA (ceDNA) vector, the method comprising: contacting a double-stranded DNA construct having a sense strand and an antisense strand with at least a first restriction endonuclease and at least a second restriction endonuclease, wherein the construct comprises a transgene expression cassette, a first non-palindromic restriction endonuclease recognition site and a corresponding first cleavage site upstream of the transgene expression cassette, and a second non-palindromic restriction endonuclease recognition site and a corresponding second cleavage site downstream of the transgene expression cassette; and wherein the first restriction endonuclease is capable of cleaving the double-stranded DNA construct at the first cleavage site, and wherein the second restriction endonuclease is capable of cleaving the double-stranded DNA construct at the second cleavage site, and wherein contacting the double-stranded DNA construct with the first restriction endonuclease and the second restriction