

Claims

1. A nucleic acid template for the cell-free, *in vitro* manufacture of single stranded nucleic acid constructs with sequestered ends, comprising a sequence encoding the following elements in a single stranded nucleic acid from 5' to 3':

- i) a first processing motif, adjacent to
- ii) a first conformational motif,
- iii) a sequence of interest,
- iv) a second conformational motif, adjacent to
- v) a second processing motif,

wherein a processing motif includes self-complementary sequences which form a base-paired section including a recognition site for an endonuclease and an associated cleavage site, and wherein a conformational motif includes at least one sequence which forms intramolecular hydrogen bonds and assumes a conformation which sequesters an end of the single stranded construct.

2. A nucleic acid template as claimed in claim 1 wherein the cleavage site within a processing motif is adjacent to a conformational motif.

3. A nucleic acid template as claimed in any preceding claim wherein the cleavage site for the endonuclease within a processing motif is at the terminal base pair of the base-paired section.

4. A nucleic acid template as claimed in any preceding claim wherein the conformational motif sequesters an end of the single stranded construct by inclusion within the assumed conformation.

5. A nucleic acid template as claimed in any preceding claim wherein the conformational motif sequesters an end of the single stranded construct by intramolecular base pairing or hydrogen bonding.

6. A nucleic acid template as claimed in any preceding claim wherein the single stranded nucleic acid construct includes any one or more of the following:

- i) an aptamer;
- ii) a nucleic acid enzyme;
- iii) a guide sequence for gene editing;

7. A nucleic acid template as claimed in any preceding claim wherein said intramolecular hydrogen bonds form between the nucleotide bases in the sequence of the conformational motif.

8. A nucleic acid template as claimed in any preceding claim wherein the intramolecular hydrogen bonds involve Watson-Crick base pairs, Hoogsteen base-pairs or non-canonical base-pairing.

9. A nucleic acid template as claimed in any preceding claim wherein a conformational motif may be a sequence which assumes one or a combination of two or more of the following conformations:

- i) quadruplex;
- ii) hairpin;
- iii) cruciform;
- iv) stem loop; and/or
- v) pseudoknot.

10. A nucleic acid template as claimed in any preceding claim wherein the sequestered end involves the intramolecular base pairing of the terminal nucleotide.

11. A nucleic acid template as claimed in any preceding claim wherein the sequestered end involves including the terminal nucleotide in an ITR structure with a double stranded D region.

12. A method of manufacturing single stranded nucleic acid molecules with sequestered ends, comprising:

(a) amplification of a circular template using a polymerase capable of rolling circle amplification, wherein said template comprises a sequence encoding the following elements in a single stranded nucleic acid:

- i) a first processing motif, adjacent to
- ii) a first conformational motif,
- iii) a sequence of interest,
- iv) a second conformational motif, adjacent to
- v) a second processing motif,

wherein a processing motif includes self-complementary sequences which form a base-paired section including a recognition site for an endonuclease containing a cleavage site, and wherein the conformational motif includes at least one sequence which forms intramolecular hydrogen bonds and assumes a conformation which sequesters the end of the single stranded nucleic acid construct, said amplification producing a single stranded nucleic acid concatemer, and

(b) processing said single stranded nucleic acid concatemer using one or more endonucleases which recognise the cleavage sites in one or more of said processing motifs.

13. A method of manufacturing single stranded nucleic acid molecules with sequestered ends as claimed in claim 12 wherein the template is as described in any one of claims 1 to 11.

14. A single stranded nucleic acid concatemer with two or more repeats of a sequence unit, said sequence unit comprising the following elements:

- i) a first processing motif, adjacent to
- ii) a first conformational motif,
- iii) a sequence of interest,
- iv) a second conformational motif, adjacent to
- v) a second processing motif,

wherein a processing motif includes self-complementary sequences which form a base-paired section including a recognition site for an endonuclease and an associated a cleavage site, and wherein the conformational motif includes at least one sequence which forms intramolecular hydrogen bonds.

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15. A method of manufacturing single stranded linear nucleic acid molecules with sequestered ends as claimed in claim 12, wherein at least one sequestered end forms a G quadruplex.

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16. A method of manufacturing single stranded linear nucleic acid molecules with sequestered ends as claimed in claim 12, wherein at least one sequestered end forms an ITR structure with a double stranded D section.