tag for easy and effective separation of said template from an RNA *in vitro* transcription reaction, it is important that this coupling is done in a directed fashion e.g. not to impair efficient run-off of the RNA polymerase (RNAP) which ensures RNA products of homogenous length (see Figure 1).

Thus, no methods are currently available which allow for a directed non-sequence, non-PCR-based coupling of a support or a tag to a linear DNA template after it is generated (e.g. after DNA preparation from an organism).

Accordingly, there is the need for providing high-quality linear DNA templates, which are associated with a support or a tag at the desired end of the linear DNA template and which can be produced at relatively low cost in large amounts so that RNA *in vitro* transcription on an industrial scale will become feasible.

Summary

In one aspect, the present disclosure refers to a linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said DNA comprises (i) a coding sequence element encoded by the coding strand in the direction of from 5' to 3' of the coding strand and (ii) an RNA polymerase promotor sequence element upstream of the coding sequence element, wherein said non-coding strand is coupled at its 3' end to a support or a tag, and wherein said support or tag is the only support or tag coupled to said DNA.

In another aspect, the present disclosure refers to a method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of: (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element; (b) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a 3' end of a strand in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of each strand; (c) incubating the DNA obtained in step (b) with a restriction endonuclease recognizing said restriction site element in order to obtain linear double stranded DNA with a modified deoxynucleotide at the 3' end of the non-coding strand; and (d) coupling the DNA obtained in step (c) via its modified deoxynucleotide to a support or a tag in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.

In yet another aspect, the present disclosure refers to a method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of: (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element; (b) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a 3' end of a strand in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of each strand; (c) coupling the DNA obtained in step (b) via the modified deoxynucleotide at the 3' end of each strand to a support or a tag; and (d) incubating the DNA obtained in step (c) with a restriction endonuclease recognizing said restriction element in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.

In yet another aspect, the present disclosure refers to a method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a tag, comprising the steps of: (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element; (b) incubating said DNA with (i) a tag-linked deoxynucleotide and (ii) an enzyme capable of adding a tag-linked deoxynucleotide at a 3'end of a strand in order to provide linear double stranded DNA with a tag- linked deoxynucleotide at the 3' end of each strand; and (c) incubating the DNA obtained in step (b) with a restriction endonuclease recognizing said restriction site element in order to obtain linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a tag, wherein the enzyme capable of adding a tag-linked deoxynucleotide at the 3' end of a strand in step (b) is selected from the group of Thermus aquaticus DNA polymerase and terminal transferase.

In yet another aspect, the present disclosure refers to a method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of: (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, wherein said DNA has a blunt end 5' of said coding element and a sticky end 3' of said coding element; (b) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a blunt end to the 3' end of a single strand and not at a sticky end in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of the non-coding strand; and (c) coupling the DNA obtained in step (b) via its modified deoxynucleotide to a support or a tag in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.

In yet another aspect, the present disclosure refers to a method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a tag, comprising the steps of: (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, wherein said DNA has a blunt end 5' of said coding element and a sticky end 3' of said coding element; and (b) incubating said DNA with (i) a tag-linked deoxynucleotide and (ii) an enzyme capable of adding a tag-linked deoxynucleotide at a blunt end to the 3' end of a single strand and not at a sticky end in order to provide linear double stranded DNA with a tag-linked deoxynucleotide at the 3' end of the non-coding strand.

In yet another aspect, the present disclosure refers to use of the linear double stranded DNA as disclosed herein in an RNA *in vitro* transcription reaction.

In yet another aspect, the present disclosure refers to a method for producing RNA in vitro comprising the steps of: (a) providing the double stranded linear DNA according to any one of claims 1 to 3 as template DNA; (b) providing (i) ribonucleoside triphosphates and (ii) a DNA-dependent RNA polymerase; and (c) incubating the DNA provided in step (a) with (i) and (ii) provided in step (b) under suitable conditions in order to produce RNA.

In yet another aspect, the present disclosure refers to a bioreactor for RNA in vitro transcription comprising (a) a reaction vessel (13) comprising the linear double stranded DNA as disclosed herein; (b) a vessel (14) comprising ribonucleoside triphosphates and DNA-dependent RNA polymerase, wherein said vessel is connected to the reaction

vessel; and (c) a product vessel (15) for collecting the RNA product, wherein said vessel is also connected to the reaction vessel.

In yet another aspect, the present disclosure refers to use of the bioreactor as disclosed herein in a method as disclosed herein.

~~In yet another aspect, the present disclosure refers to a kit comprising (a) a modified deoxynucleotide; (b) a *Thermus aquaticus* DNA polymerase capable of adding said modified deoxynucleotide to the 3' end of a strand at a blunt DNA end; (c) an activated support or tag; (d) a counterpart of said support or tag associating in a highly specific manner with said support or tag.~~

~~In yet another aspect, the present disclosure refers to a kit comprising (a) a tag-linked deoxynucleotide; (b) a *Thermus aquaticus* DNA polymerase capable of adding said tag-linked deoxynucleotide to the 3' end of a strand at a blunt DNA end; (c) a counterpart of said tag associating in a highly specific manner with said tag~~

The present disclosure solves the above needs, *inter alia* by providing linear double stranded DNA as described below in a first example of the disclosure and by providing methods of producing said linear double stranded DNA as described in examples 2A-2D. In a third and fourth example, the present disclosure relates to the use of the DNA of example 1 of the present disclosure for RNA *in vitro* transcription and to a method for producing RNA *in vitro* comprising the use of the DNA of example 1. The present disclosure furthermore relates to a bioreactor for RNA *in vitro* transcription and a kit comprising parts which enables a person to produce the linear double stranded DNA of example 1 of the disclosure according to the methods of examples 2A-2D of the disclosure.

All examples mentioned in the following chapters relate to the specific example of this chapter.

<u>First example: Linear double stranded DNA coupled to a single support or tag at the 3' end of its non-coding strand</u>

In a first example, the present disclosure relates to a linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said DNA comprises a coding sequence element encoded by the coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, and wherein said support or tag is the only support or tag coupled to said DNA.

In a particularly preferred example, the present disclosure relates to a linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said DNA comprises a coding sequence element encoded by the coding strand, wherein said non-coding strand is coupled at its 3' end to a tag, and wherein the tag is the only tag coupled to said DNA.

In a specific example, the non-coding strand coupled at its 3' end to a support or tag has at least one, preferably exactly one, deoxynucleotide overhang compared to 5' end of the complementary coding strand. Suitably, the at least one deoxynucleotide overhang is at least one deoxyadenosine, in particular exactly one deoxyadenosine.

C L A I M S

1. A linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said DNA comprises (i) a coding sequence element encoded by the coding strand in the direction of from 5' to 3' of the coding strand and (ii) an RNA polymerase promotor sequence element upstream of the coding sequence element, wherein said non-coding strand is coupled at its 3' end to a support or a tag, and wherein said support or tag is the only support or tag coupled to said DNA.

2. The linear double stranded DNA according to claim 1, wherein said non-coding strand is coupled at its 3' end to a support or a tag via a triazole.

3. The linear double stranded DNA according to claim 1 or 2, wherein said tag is biotin, preferably wherein said biotin is associated with streptavidin, preferably a streptavidin coated bead, most preferably a streptavidin coated magnetic bead.

4. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of:
   (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element;
   (b) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a 3' end of a strand in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of each strand;
   (c) incubating the DNA obtained in step (b) with a restriction endonuclease recognizing said restriction site element in order to obtain linear double stranded DNA with a modified deoxynucleotide at the 3' end of the non-coding strand; and
   (d) coupling the DNA obtained in step (c) via its modified deoxynucleotide to a support or a tag in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.

5. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of:

(a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element;

(b) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a 3' end of a strand in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of each strand;

(c) coupling the DNA obtained in step (b) via the modified deoxynucleotide at the 3' end of each strand to a support or a tag; and

(d) incubating the DNA obtained in step (c) with a restriction endonuclease recognizing said restriction element in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.

6. The method according to claim 4 or 5, wherein the enzyme capable of adding a modified deoxynucleotide at the 3' end of a strand in step (b) is a DNA polymerase, preferably .
wherein the DNA polymerase is selected from the group consisting of a *Thermus aquaticus* DNA polymerase, an *Escherichia coli* DNA polymerase, a *Saccharomyces cerevisiae* DP1 DNA polymerase, a mammalian DNA β polymerase, an engineered DNA polymerase, a DNA polymerase I large (Klenow) fragment and a terminal transferase.

7. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a tag, comprising the steps of:

(a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element;

(b) incubating said DNA with (i) a tag-linked deoxynucleotide and (ii) an enzyme capable of adding a tag-linked deoxynucleotide at a 3'end of a strand in order to provide linear double stranded DNA with a tag- linked deoxynucleotide at the 3' end of each strand; and

(c) incubating the DNA obtained in step (b) with a restriction endonuclease recognizing said restriction site element in order to obtain linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a tag,

wherein the enzyme capable of adding a tag-linked deoxynucleotide at the 3' end of a strand in step (b) is selected from the group of *Thermus aquaticus* DNA polymerase and terminal transferase.

8. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of:

(a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, wherein said DNA has a blunt end 5' of said coding element and a sticky end 3' of said coding element;

(b) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a blunt end to the 3' end of a single strand and not at a sticky end in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of the non-coding strand; and

(c) coupling the DNA obtained in step (b) via its modified deoxynucleotide to a support or a tag in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.

9. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a tag, comprising the steps of:

(a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, wherein said DNA has a blunt end 5' of said coding element and a sticky end 3' of said coding element; and

(b) incubating said DNA with (i) a tag-linked deoxynucleotide and (ii) an enzyme capable of adding a tag-linked deoxynucleotide at a blunt end to the 3' end of a single strand and not at a sticky end in order to provide linear double stranded DNA with a tag-linked deoxynucleotide at the 3' end of the non-coding strand.

10. Use of the linear double stranded DNA according to any one of claims 1 to 3 in an RNA *in vitro* transcription reaction.

11. A method for producing RNA *in vitro* comprising the steps of:

(a) providing the double stranded linear DNA according to any one of claims 1 to 3 as template DNA;

(b) providing (i) ribonucleoside triphosphates and (ii) a DNA-dependent RNA polymerase; and

(c) incubating the DNA provided in step (a) with (i) and (ii) provided in step (b) under suitable conditions in order to produce RNA.

12. A bioreactor for RNA *in vitro* transcription comprising

(a) a reaction vessel (13) comprising the linear double stranded DNA according to any one of claims 1 to 3;

(b) a vessel (14) comprising ribonucleoside triphosphates and DNA-dependent RNA polymerase, wherein said vessel is connected to the reaction vessel; and

(c) a product vessel (15) for collecting the RNA product, wherein said vessel is also connected to the reaction vessel.

13. Use of the bioreactor according to claim 12 in a method according to claim 11.

~~14. A kit comprising~~

~~(a) a modified deoxynucleotide;~~

~~(b) a *Thermus aquaticus* DNA polymerase capable of adding said modified deoxynucleotide to the 3' end of a strand at a blunt DNA end;~~

~~(c) an activated support or tag;~~

~~(d) a counterpart of said support or tag associating in a highly specific manner with said support or tag.~~

~~15. A kit comprising~~

~~(a) a tag-linked deoxynucleotide;~~

~~(b) a *Thermus aquaticus* DNA polymerase capable of adding said tag-linked deoxynucleotide to the 3' end of a strand at a blunt DNA end;~~

~~(c) a counterpart of said tag associating in a highly specific manner with said tag.~~