CLAIMS

1. Method for *in vitro* transcription of a linear template DNA, comprising the steps of:

a) contacting a plasmid DNA having at least one recognition sequence for a restriction enzyme with said restriction enzyme, wherein the restriction enzyme is immobilized to a solid support, thereby linearizing the plasmid DNA and providing the linear template DNA; and

b) transcribing the linear template DNA into RNA *in vitro*, and

c) obtaining the resulting RNA.

2. Method according to claim 1, wherein the restriction enzyme is a type II restriction enzyme.

3. Method according to claim 2, wherein the restriction enzyme is selected from the group consisting of EcoRI, BciVI, XbaI, NdeI, AflII, SacI, KpnI, SmaI, BamHI, XbaI, SalI, SbfI, PstI and HindIII.

4. Method according to any of the preceding claims, wherein the restriction enzyme is immobilized by covalent binding.

5. Method according to claim 4, wherein the covalent binding is a disulfide bridge or a thioether bond.

6. Method according to claim 4 or 5, wherein the restriction enzyme is immobilized by covalent binding to a thiol-activated support material, haloacetyl functionalized solid support, pyridyl disulfide-functionalized solid support, maleimide-activated solid support, epoxy activated solid support or a mixture thereof.

7. Method according to claim 6, wherein the restriction enzyme is bound to the thiol-activated support material via a thiol group of at least one cysteine residue.

8. Method according to claim 7, wherein the at least one cysteine residue which is bound to the thiol-activated support material is not present in the wild-type restriction enzyme.

9. Method according to claim 7 or 8, wherein the at least one cysteine residue which is bound to the thiol-activated support material has been introduced by substituting an amino acid within the wild-type restriction enzyme with cysteine or by inserting a cysteine into the wild-type restriction enzyme, preferably by adding a cysteine residue to the N or C terminus of the restriction enzyme.

10. Method according to any of the preceding claims, wherein the restriction enzyme is selected from the group consisting of:

a) EcoRI according to SEQ ID No. 1, wherein the lysine on position 277 of SEQ ID No. 1 is replaced with cysteine or a functional variant of EcoRI having at least 80% sequence identity to SEQ ID No. 1, wherein the lysine on a position corresponding to position 277 of SEQ ID No. 1 is replaced with cysteine;

b) EcoRI according to SEQ ID No.1 or a functional variant thereof having at least 80% sequence identity to SEQ ID No. 1, wherein a cysteine is added to the C-terminus, preferably via a linker;

c) HindIII according to SEQ ID No. 2, wherein the leucine on position 300 of SEQ ID No. 2 is substituted with cysteine or a functional variant of HindIII having at least 80% sequence identity to SEQ ID No. 2, wherein the leucine on a position corresponding to position 300 of SEQ ID No. 2 is substituted with cysteine;

d) HindIII according to SEQ ID No. 2, wherein a cysteine residue is added to the C-terminus, preferably via a linker, or a functional variant of HindIII

having at least 80% sequence identity to SEQ ID No. 2, wherein a cysteine residue is added to the C-terminus, preferably via a linker; and

e) BciVI according to SEQ ID No. 3, wherein a cysteine residue is added to the C-terminus, preferably via a linker or a functional variant thereof having at least 80% sequence identity to SEQ ID No. 3, wherein a cysteine residue is added to the C-terminus, preferably via a linker.

11. Method according to claim 10, wherein the linker has the sequence GGGGSGGGGS (SEQ ID No. 49).

12. Method according to any of claims 8 to 11, wherein, if present, one or more cysteine residues in the wild-type restriction enzyme are replaced with another amino acid.

13. Method according to claim 12, wherein the restriction enzyme is selected from the group consisting of:

a) EcoRI according to SEQ ID No. 1, wherein the cysteine position 218 of SEQ ID No. 1 is replaced with another amino acid or a functional variant of EcoRI having at least 80% sequence identity to SEQ ID No. 1, wherein the cysteine on a position corresponding to position 218 of SEQ ID No. 1 is replaced with another amino acid; or

b) BciVI according to SEQ ID No. 3, wherein the cysteine on position 271 of SEQ ID No. 3 is replaced with another amino acid or a functional variant of BciVI having at least 80% sequence identity to SEQ ID No. 3, wherein the cysteine on a position corresponding to position 271 of SEQ ID No. 3 is replaced with another amino acid.

14. Method according to any of the preceding claims, wherein the restriction enzyme is selected from the group consisting of:

a) EcoRI according to SEQ ID No. 1, wherein the lysine on position 277 of SEQ ID No. 1 is replaced with cysteine and the cysteine on position 218 of SEQ ID No. 1 is replaced with alanine, valine or serine or a functional variant of

EcoRI having at least 80% sequence identity to SEQ ID No. 1, wherein the lysine on a position corresponding to position 277 of SEQ ID No. 1 is replaced with cysteine and the cysteine on a position corresponding to position 218 of SEQ ID No. 1 is replaced with alanine, valine or serine;

b) EcoRI according to SEQ ID No.1, wherein a cysteine is added to the C-terminus via a linker comprising 12 glycine residues and the cysteine on position 218 of SEQ ID No. 1 is replaced with alanine, valine or serine or a functional variant of EcoRI having at least 80% sequence identity to SEQ ID No. 1 wherein a cysteine is added to the C-terminus via a linker comprising 12 glycine residues and the cysteine on a position corresponding to position 218 of SEQ ID No. 1 is replaced with alanine, valine or serine;

c) EcoRI according to SEQ ID No.1, wherein a cysteine is added to the C-terminus via a linker having the sequence GGGGSGGGGS and the cysteine on position 218 of SEQ ID No. 1 is replaced with alanine, valine or serine or a functional variant of EcoRI having at least 80% sequence identity to SEQ ID No. 1 wherein a cysteine is added to the C-terminus via a linker having the sequence GGGGSGGGGS and the cysteine on a position corresponding to position 218 of SEQ ID No. 1 is replaced with alanine, valine or serine

d) BciVI according to SEQ ID No. 3, wherein a cysteine residue is added to the C-terminus and the cysteine on position 271 of SEQ ID No. 3 is replaced with serine, valine or alanine or a functional variant of BciVI having at least 80% sequence identity to SEQ ID No. 3, wherein a cysteine residue is added to the C-terminus and the cysteine on a position corresponding to position 271 of SEQ ID No. 3 is replaced with serine, valine or alanine.

15. Method according to any of the preceding claims, wherein the restriction endonuclease further comprises a purification tag.

16. Method according to claim 15, wherein the purification tag comprises six histidine residues.

17. Method according to any of the preceding claims, wherein the method is performed in an enzyme reactor.

18. Method according to any of the preceding claims, further comprising a step of purifying the linear template DNA after step a).

19. Method according to claim 18, wherein the linear template DNA is purified by filtration.

20. Kit comprising:
a restriction endonuclease which is immobilized onto a solid support, and
a suitable restriction buffer, further comprising a RNA polymerase, a nucleotide mixture and an *in vitro* transcription buffer.