Claims

1. A method for the determination of the binding of a protein to a nucleic acid molecule comprising a nucleic acid sequence, said method comprising the steps of:

   a) denaturing a double-stranded nucleic acid molecule comprising a nucleic acid sequence by applying a physical force to the said molecule;
   b) providing the said protein;
   c) renaturing the said double stranded nucleic acid molecule in the presence of the said protein;
   d) detecting a blockage of the re-naturation of the double-stranded nucleic acid; and
   e) determining the position of the said blockage on the said double-stranded nucleic acid molecule.

2. The method of claim 1, wherein a single-stranded nucleic acid molecule corresponding to the said sequence is further provided in step b).

3. The method of any of claim 1 or 2, wherein the said double-stranded nucleic acid molecule is a hairpin.

4. The method of any one of claims 1 to 3, wherein at least one of the bases of one of the strands of the double-stranded nucleic acid is attached directly or indirectly to a surface, and wherein at least one of the bases of the other strand of the double-stranded nucleic acid is attached to a movable surface.

5. The method of any one of claims 1 to 4, wherein the double-stranded nucleic acid is denatured in step a) by moving away the supports.

6. The method of claim 5, wherein a physical force above or equal to 15 pN, preferably above or equal to 17 pN, more preferably above or equal to 18 pN, is applied to the double-stranded molecule by moving away the supports.

7. The method of any one of claims 1 to 6, wherein the denatured double-stranded nucleic acid is re-natured in step c) by bringing the supports together.

8. The method of claim 7, wherein the force applied to the double-stranded molecule is reduced to less than or equal to 12 pN, preferably less than or equal to 11 pN, more preferably less than or equal to 10 pN, by bringing the supports together.

9. The method of any one of claims 1 to 8, wherein the ends of the double-stranded nucleic acid which are not attached to a support are joined to one another covalently or not covalently.

10. The method of any one of claims 1 to 9, wherein the steps a) to d) are repeated several times (so as to accumulate measurements and increase the signal/noise ratio).

11. The method of any one of claims 1 to 10, wherein the detection of step d) comprises measuring the distance (z) between the two ends of the double-stranded nucleic acid molecule which are attached to the support.

12. The method of claim 11, comprising a further step of measuring the distance (z_{high}) between the two ends of the double-stranded nucleic acid molecules which are attached to the support, when the said double-stranded nucleic acid molecule is denatured.

13. The method of claim 12, wherein step e) further comprises the prior step ofcomparing z and z_{high}.

14. The method of any one of claims 1 to 13, comprising a further step of measuring the duration of the blockage.

15. The method of claim 14, comprising a further step of comparing the duration of the blockage with a reference value.

16. The method of anyone of claims 1 to 15, further comprising a step of sequencing the nucleic acid sequence bound by the said protein.

17. A method for identifying nucleic acid molecules comprising a sequence capable of binding a specific nucleic acid-binding protein, said method comprising the steps of:
    a) providing a population of double-stranded nucleic acid molecules;
    b) testing the binding of the said protein to the said nucleic acid molecule by the method of any one of claims 1 to 16; and
    c) selecting the nucleic acid molecules capable of binding the said protein.

18. A method for detecting at least one modified base comprised within a double-stranded nucleic acid molecule, said method comprising the steps of:
    a) providing the said double-stranded nucleic acid;
    b) providing a protein capable of binding said modified base; and
    c) testing the binding of the said protein to the said nucleic acid molecule by the method of any one of claims 1 to 16.

19. The method of claim 18, wherein the modified base is selected in the group consisting of 4-methylcytosine (4mC), 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) 5-carboxylcytosine (5caC), 5-hydroxymethyluracil (5hmU), and N6-methyladenosine (m6A).

20. The method of anyone of claims 18 or 19, wherein the modified base is 5mC.

21. The method of anyone of claims 18 to 20, wherein the said protein is an antibody capable of binding said modified base.

22. A method for detecting at least one mismatch in a double-stranded nucleic acid molecule, said method comprising the steps of:
    a) providing the said double-stranded nucleic acid molecule;
    b) providing a protein capable of binding a mismatched base; and
    c) testing the binding of the said protein to the said nucleic acid molecule by the method of any one of claims 1 to 16.

23. The method of claim 22, wherein the said protein is selected from the group consisting of a MutS dimer, Msh2/Msh6 (MutSα), and Msh2/Msh3 (MutSß).

24. A method for detecting a SNP in a sequence contained in a nucleic acid molecule, said method comprising the steps of:
    a) hybridizing the said nucleic acid with a single-stranded nucleic acid comprising the sequence found in the majority of the population; and
    b) detecting the resulting mismatch by the method of any one of claims 22 or 23.

25. A method for identifying at least one compound capable of preventing the interaction between a protein and its binding site, said method comprising the steps of:
    a) providing the said protein and a nucleic acid molecule comprising a sequence corresponding to the said binding site;
    b) providing a compound; and
    c) testing the binding of the said protein to the said nucleic acid molecule by the method of any one of claims 1 to 16.

Claims

1. A method for the determination of the binding of a protein to a nucleic acid molecule comprising a nucleic acid sequence, said method comprising the steps of:

   a) denaturing a double-stranded nucleic acid molecule comprising a nucleic acid sequence by applying a physical force to the said molecule;
   b) providing the said protein;
   c) renaturing the said double stranded nucleic acid molecule in the presence of the said protein;
   d) detecting a blockage of the re-naturation of the double-stranded nucleic acid; and
   e) determining the position of the said blockage on the said double-stranded nucleic acid molecule.

2. The method of claim 1, wherein a single-stranded nucleic acid molecule corresponding to the said sequence is further provided in step b).

3. The method of any of claim 1 or 2, wherein the said double-stranded nucleic acid molecule is a hairpin.

4. The method of any one of claims 1 to 3, wherein at least one of the bases of one of the strands of the double-stranded nucleic acid is attached directly or indirectly to a support, and wherein at least one of the bases of the other strand of the double-stranded nucleic acid is attached to a movable support.

5. The method of claim 4, wherein the double-stranded nucleic acid is denatured in step a) by moving away the supports.

6. The method of claim 5, wherein a physical force above or equal to 15 pN, preferably above or equal to 17 pN, more preferably above or equal to 18 pN, is applied to the double-stranded molecule by moving away the supports.

7. The method of claim 5 or 6, wherein the denatured double-stranded nucleic acid is re-natured in step c) by bringing the supports together.

8. The method of claim 7, wherein the force applied to the double-stranded molecule is reduced to less than or equal to 12 pN, preferably less than or equal to 11 pN, more preferably less than or equal to 10 pN, by bringing the supports together.

9. The method of any one of claims 4 to 8, wherein the ends of the double-stranded nucleic acid which are not attached to the support are joined to one another covalently or not covalently.

10. The method of any one of claims 1 to 9, wherein the steps a) to d) are repeated several times (so as to accumulate measurements and increase the signal/noise ratio).

11. The method of any one of claims 4 to 10, wherein the detection of step d) comprises measuring the distance (z) between the two ends of the double-stranded nucleic acid molecule which are attached to the support.

12. The method of claim 11, comprising a further step of measuring the distance (z_{high}) between the two ends of the double-stranded nucleic acid molecules which are attached to the support, when the said double-stranded nucleic acid molecule is denatured.

13. The method of claim 12, wherein step e) further comprises the prior step of comparing z and z_{high}.

14. The method of any one of claims 1 to 13, comprising a further step of measuring the duration of the blockage.

15. The method of claim 14, comprising a further step of comparing the duration of the blockage with a reference value.

16. The method of anyone of claims 1 to 15, further comprising a step of sequencing the nucleic acid sequence bound by the said protein.

17. A method for identifying nucleic acid molecules comprising a sequence capable of binding a specific nucleic acid-binding protein, said method comprising the steps of:
    a) providing a population of double-stranded nucleic acid molecules;
    b) testing the binding of the said protein to the said nucleic acid molecule by the method of any one of claims 1 to 16; and
    c) selecting the nucleic acid molecules capable of binding the said protein.

18. A method for detecting at least one modified base comprised within a double-stranded nucleic acid molecule, said method comprising the steps of:
    a) providing the said double-stranded nucleic acid;
    b) providing a protein capable of binding said modified base; and
    c) testing the binding of the said protein to the said nucleic acid molecule by the method of any one of claims 1 to 16.

19. The method of claim 18, wherein the modified base is selected in the group consisting of 4-methylcytosine (4mC), 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) 5-carboxylcytosine (5caC), 5-hydroxymethyluracil (5hmU), and N6-methyladenosine (m6A).

20. The method of anyone of claims 18 or 19, wherein the modified base is 5mC.

21. The method of anyone of claims 18 to 20, wherein the said protein is an antibody capable of binding said modified base.

22. A method for detecting at least one mismatch in a double-stranded nucleic acid molecule, said method comprising the steps of:
    a) providing the said double-stranded nucleic acid molecule;
    b) providing a protein capable of binding a mismatched base; and
    c) testing the binding of the said protein to the said nucleic acid molecule by the method of any one of claims 1 to 16.

23. The method of claim 22, wherein the said protein is selected from the group consisting of a MutS dimer, Msh2/Msh6 (MutSα), and Msh2/Msh3 (MutSβ).

24. A method for detecting a SNP in a sequence contained in a nucleic acid molecule, said method comprising the steps of:
    a) hybridizing the said nucleic acid with a single-stranded nucleic acid comprising the sequence found in the majority of the population; and
    b) detecting the resulting mismatch by the method of any one of claims 22 or 23.

25. A method for identifying at least one compound capable of preventing the interaction between a protein and its binding site, said method comprising the steps of:
    a) providing the said protein and a nucleic acid molecule comprising a sequence corresponding to the said binding site;
    b) providing a compound; and
    c) testing the binding of the said protein to the said nucleic acid molecule by the method of any one of claims 1 to 16.