CLAIMS

1. A nucleic acid sensor system comprising a first and second single stranded DNA sensor parts which, when ligated together generate a single strand of a DNA expression cassette that comprises:

(i) a promoter;

(ii) a ribosome binding site (RBS); and

(iii) a coding sequence;

in which a target nucleic acid hybridization sequence is located within the first and second single stranded DNA sensor parts.

2. A nucleic acid sensor system according to Claim 1, in which the target nucleic acid hybridization sequence comprises 3' and 5' hybridization regions, in which the 3' hybridization region is included in the first sensor part and the 5' hybridization region is included in the second sensor part so that, when the sensor system is contacted with a sample that includes a target nucleic acid that hybridizes with the target nucleic acid sequence, hybridization with the target nucleic acid enables ligation of the first and second parts to generate the single strand.

3. A nucleic acid sensor system according to Claim 1 or 2, in which the DNA expression cassette is a non-template DNA expression cassette.

4. A nucleic acid sensor system according to Claim 1, 2 or 3, in which the first and second sensor parts of the expression cassette are separated at any given position within the expression cassette.

5. A nucleic acid sensor system according to Claim 4, in which the separation occurs within the target nucleic acid hybridization sequence.

6. A nucleic acid sensor system according to any of Claims 1 to 4, in which the target nucleic acid hybridization sequence is located at any given position within the expression cassette.

7. A nucleic acid sensor system according to any preceding claim, in which either the first or the second sensor part comprises:

(a) the coding sequence and the remaining sensor part comprises the promoter;

(b) the promoter and the ribosome binding site and the remaining sensor part comprises the coding sequence;

(c) the promoter, the ribosome binding site, and a start codon of the coding sequence and the remaining sensor part comprises the remaining coding sequence;

(d) the promoter and the remaining sensor part comprises the ribosome binding site and the coding sequence; or

(e) the promoter, the ribosome binding site, and a 5' portion of the coding sequence and the remaining sensor part comprises the remaining coding sequence.

8. A nucleic acid sensor system according to Claim 7, in which the first sensor part comprises, from 5' to 3':

(i) the promoter;

(ii) the ribosome binding site (RBS);

(iii) the start codon for the coding sequence; and

(iv) the 3' hybridization region in the form of a reading frame in-frame with the start codon;

and the second sensor part comprises from 5' to 3':

(i) the 5' hybridization region in the form of a reading frame in-frame with the remaining coding sequence; and

(ii) a remaining coding sequence linked downstream of and in-frame with the 5' hybridization region.

9. A nucleic acid sensor system according to Claim 7, in which the first sensor part comprises, from 5' to 3':

(i) the promoter; and

(ii) the 3’ hybridization region;

and the second sensor part comprises, from 5’ to 3’:

(i) the 5’ hybridization region;

(ii) the ribosome binding sequence; and

(iii) the coding sequence.

10. A nucleic acid sensor system according to Claim 7, in which the first sensor part comprises, from 5’ to 3’:

(i) the promoter;

(ii) the RBS;

(iii) a first portion of the coding sequence, comprising a start codon and at least one additional codon;

(iv) the 3’ hybridization region in the form of a reading frame in-frame with the start codon;

and the second sensor part comprises, from 5’ to 3’:

(i) the 5’ hybridization region in the form of a reading frame in-frame with the first portion of the coding sequence and in-frame with the second portion of the coding sequence; and

(ii) a second portion of the coding sequence linked downstream of and in-frame with the 5’ hybridization region.

11. A nucleic acid sensor system according to any preceding claim, in which at least a portion of the 3’ hybridization region or the 5’ hybridization region is in the promoter, the ribosome binding site, or the coding sequence.

12. A nucleic acid sensor system according to any preceding claim, in which the 3’ hybridization region or the 5’ hybridization region is not within the promoter, the ribosome binding site, or the coding sequence.

13. A nucleic acid sensor system according to any preceding claim, in which the 3’ hybridization region is 3’ of any promoter, ribosome binding site, or coding sequence in the first sensor part.

14. A nucleic acid sensor system according to any preceding claim, in which the 5’ hybridization region is 5’ of any promoter, ribosome binding site, or coding sequence in the second sensor part.

15. A nucleic acid sensor system according to any preceding claim in which the 3’ hybridization region and the 5’ hybridization region are each at least 6 nucleotides in length.

16. A nucleic acid sensor system according to any preceding claim, in which the first and second sensor parts are on the same molecule.

17. A nucleic acid sensor system according to any preceding claim, in which the 5’ end of the first sensor part is linked to the 3’ end of the second sensor part through intervening ssDNA sequences so that the first and second sensor part form a single molecule.

18. A nucleic acid sensor system according to any preceding claim, in which the system further comprises a primer complementary to a sequence within the non-template expression cassette or intervening ssDNA sequences.

19. A nucleic acid sensor system according to any preceding claim, in which the first and second sensor parts are on at least two separate molecules.

20. A method for detecting a target nucleic acid in a sample, comprising:

(a) providing a sample comprising the target nucleic acid;

(b) contacting sample comprising the target nucleic acid with the nucleic acid sensor system according to any preceding claim in the presence of a ligase under conditions favourable to the hybridization of the target nucleic acid to the 3’ hybridization region of the first sensor part and to the 5’ hybridization region of the second sensor part of the expression cassette, to thereby generate a reaction product comprising the target nucleic acid hybridized to the first sensor part and the second sensor part operably-linked to each other;

(c) contacting the reaction product produced in step (b) with a cell-free expression system in the presence of a strand displacing DNA Polymerase and a primer, under conditions favourable to the production of a reporter protein;

(d) contacting reaction product produced in step (c) with a reagent enabling the detection of the expression of the reporter protein;

(e) measuring the expression of the reporter protein produced in step (d) to determine the presence and/or amount of the target nucleic acid in the sample.

CLAIMS

1. A nucleic acid sensor system comprising a first and second single stranded DNA sensor parts which, when ligated together generate a single strand of a DNA expression cassette that comprises:

(i) a promoter;

(ii) a ribosome binding site (RBS); and

(iii) a coding sequence of a polypeptide;

in which a target nucleic acid hybridization sequence is located within the first and second single stranded DNA sensor parts, in which the target nucleic acid hybridization sequence comprises 3' and 5' hybridization regions, in which the 3' hybridization region is included in the first sensor part and the 5' hybridization region is included in the second sensor part so that, when the sensor system is contacted with a sample that includes a target nucleic acid that hybridizes with the target nucleic acid hybridization sequence, hybridization of the target nucleic acid hybridization sequence with the target nucleic acid enables ligation of the first and second parts to generate the single strand, and in which the first and second sensor parts of the expression cassette are separated within the target nucleic acid hybridization sequence.

2. A nucleic acid sensor system according to Claim 1, in which the DNA expression cassette is a non-template DNA expression cassette.

3. A nucleic acid sensor system according to Claim 1 or 2, in which the target nucleic acid hybridization sequence is located at any given position within the expression cassette.

4. A nucleic acid sensor system according to any preceding claim, in which either the first or the second sensor part comprises:

(a) the coding sequence and the remaining sensor part comprises the promoter;

(b) the promoter and the ribosome binding site and the remaining sensor part comprises the coding sequence;

(c) the promoter, the ribosome binding site, and a start codon of the coding sequence and the remaining sensor part comprises the remaining coding sequence;

(d) the promoter and the remaining sensor part comprises the ribosome binding site and the coding sequence; or

(e) the promoter, the ribosome binding site, and a 5’ portion of the coding sequence and the remaining sensor part comprises the remaining coding sequence.

5. A nucleic acid sensor system according to Claim 4, in which the first sensor part comprises, from 5’ to 3’:

(i) the promoter;

(ii) the ribosome binding site (RBS);

(iii) the start codon for the coding sequence; and

(iv) the 3’ hybridization region in the form of a reading frame in-frame with the start codon;

and the second sensor part comprises from 5’ to 3’:

(i) the 5’ hybridization region in the form of a reading frame in-frame with the remaining coding sequence; and

(ii) a remaining coding sequence linked downstream of and in-frame with the 5’ hybridization region.

6. A nucleic acid sensor system according to Claim 4, in which the first sensor part comprises, from 5’ to 3’:

(i) the promoter; and

(ii) the 3’ hybridization region;

and the second sensor part comprises, from 5’ to 3’:

(i) the 5’ hybridization region;

(ii) the ribosome binding sequence; and

(iii) the coding sequence.

7. A nucleic acid sensor system according to Claim 4, in which the first sensor part comprises, from 5’ to 3’:

(i) the promoter;

(ii) the RBS;

(iii) a first portion of the coding sequence, comprising a start codon and at least one additional codon;

(iv) the 3’ hybridization region in the form of a reading frame in-frame with the start codon;

and the second sensor part comprises, from 5’ to 3’:

(i) the 5’ hybridization region in the form of a reading frame in-frame with the first portion of the coding sequence and in-frame with the second portion of the coding sequence; and

(ii) a second portion of the coding sequence linked downstream of and in-frame with the 5’ hybridization region.

8. A nucleic acid sensor system according to any preceding claim, in which at least a portion of the 3’ hybridization region or the 5’ hybridization region is in the promoter, the ribosome binding site, or the coding sequence.

9. A nucleic acid sensor system according to any preceding claim, in which the 3’ hybridization region or the 5’ hybridization region is not within the promoter, the ribosome binding site, or the coding sequence.

10. A nucleic acid sensor system according to any preceding claim, in which the 3’ hybridization region is 3’ of any promoter, ribosome binding site, or coding sequence in the first sensor part.

11. A nucleic acid sensor system according to any preceding claim, in which the 5’ hybridization region is 5’ of any promoter, ribosome binding site, or coding sequence in the second sensor part.

12. A nucleic acid sensor system according to any preceding claim, in which the 3’ hybridization region and the 5’ hybridization region are each at least 6 nucleotides in length.

13. A nucleic acid sensor system according to any preceding claim, in which the first and second sensor parts are on the same molecule.

14. A nucleic acid sensor system according to any preceding claim, in which the 5’ end of the first sensor part is linked to the 3’ end of the second sensor part through intervening ssDNA sequences so that the first and second sensor part form a single molecule.

15. A nucleic acid sensor system according to Claim 2 or 14, in which the system further comprises a primer complementary to a sequence within the non-template expression cassette or intervening ssDNA sequences.

16. A nucleic acid sensor system according to any preceding claim, in which the first and second sensor parts are on at least two separate molecules.

17. A nucleic acid system according to any preceding claim, in which the polypeptide is a reporter protein.

18. A nucleic acid system according to Claim 17, in which the reporter protein is selected from the group consisting of: a luciferase, nanoluciferase, beta-lactamase, beta-galactosidase, horseradish peroxidase, alkaline phosphatase, catalase, carbonic anhydrase, green fluorescent protein, red fluorescent protein, cyan fluorescent protein, yellow fluorescent protein, trypsin, a protease, a peptide that complements and activates a truncated reporter protein, and a polypeptide that is detectable by an assay.

19. A method for detecting a target nucleic acid in a sample, comprising:

(a) providing a sample comprising the target nucleic acid;

(b) contacting sample comprising the target nucleic acid with the nucleic acid sensor system according to any preceding claim in the presence of a ligase under conditions favourable to the hybridization of the target nucleic acid to the 3’ hybridization region of the first sensor part and to the 5’ hybridization region of the second sensor part of the expression cassette, to thereby generate a reaction product comprising the target nucleic acid hybridized to the first sensor part and the second sensor part operably-linked to each other;

(c) contacting the reaction product produced in step (b) with a cell-free expression system in the presence of a strand displacing DNA Polymerase and a primer, under conditions favourable to the production of a reporter protein;

(d) contacting reaction product produced in step (c) with a reagent enabling the detection of the expression of the reporter protein;

(e) measuring the expression of the reporter protein produced in step (d) to determine the presence and/or amount of the target nucleic acid in the sample.