

CLAIMS

1. A pair of non-naturally occurring nucleic acid probes for detecting a polynucleotide analyte, comprising:
 - i. a first nucleic acid probe comprising:
 - a) a first probe binding arm that is complementary to a first probe target region of a bridge probe; and
 - b) a first polynucleotide analyte binding arm that is complementary to a first analyte target region of a polynucleotide analyte, and
 - ii. a second nucleic acid probe comprising:
 - a) a second probe binding arm that is complementary to a second probe target region of the bridge probe; wherein the first probe target region is located downstream of the second probe target region on the bridge probe, and
 - b) a second polynucleotide analyte binding arm that is complementary to a second analyte target region of the polynucleotide analyte, wherein the second analyte target region is located downstream of the first analyte target region on the polynucleotide analyte,

wherein binding of the first polynucleotide analyte binding arm to the first analyte target region and binding of the second polynucleotide analyte binding arm to the second analyte target region permit binding of the first probe binding arm to the first bridge probe target region and binding of the second probe binding arm to the second bridge probe target region, thereby detecting the polynucleotide analyte.
2. The pair of non-naturally occurring nucleic acid probes of claim 1, wherein the polynucleotide analyte binding arm in the first and/or second nucleic acid probe consists of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides.
3. The pair of non-naturally occurring nucleic acid probes of claim 1 or 2, wherein the probe binding arm in the first and/or second nucleic acid probes consists of 9 or 10 nucleotides.

4. The pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 3, wherein the probe binding arm in the first and/or second nucleic acid probes comprises an identification portion for binding to a unique bridge probe.
5. The pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 4, wherein the first and second nucleic acid probes comprise a linker positioned between the probe binding arm and the polynucleotide analyte binding arm.
6. The pair of non-naturally occurring nucleic acid probes of claim 5, wherein the linker consists of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleobases.
7. The pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 6, wherein the bridge probe is a readout probe that is coupled or conjugated to a label (such as a fluorescent label).
8. The pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 6, wherein the bridge probe is detected via hybridization to a readout probe that is conjugated to a label (such as a fluorescent label).
9. The pair of non-naturally occurring nucleic acid probes of claim 8, wherein the readout probe hybridizes to a terminal region of the bridge probe.
10. The pair of non-naturally occurring nucleic acid probes of claim 8, wherein the readout probe hybridizes to a central region of the bridge probe.
11. The pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 10, wherein the first analyte target region is immediately adjacent to the second analyte target region.
12. The pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 11, wherein the first analyte target region is spaced from the second analyte target region by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleobases.

13. The pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 12, wherein the first probe target region is immediately adjacent to the second probe target region.
14. The pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 13, wherein the first probe target region is spaced from the second probe target region by no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 nucleobases.
15. A probe system comprising a pair of non-naturally occurring nucleic acid probes of any one of claims 1 to 14.
16. The probe system of claim 15, wherein the probe system further comprises a bridge probe.
17. A method of detecting a polynucleotide analyte in a sample, the method comprising:
 - (a) contacting the sample with a pair of non-naturally occurring nucleic acid probes according to any one of claims 1 to 14 or a probe system of claim 15 or 16; and
 - (b) detecting the polynucleotide analyte based on hybridization to a unique bridge probe in the presence of the polynucleotide analyte.
18. A library for detecting two or more polynucleotide analytes in a sample; the library comprising two or more pairs of non-naturally occurring nucleic acid probes according to any one of claims 1 to 14 or a plurality of probe systems according to claim 15 or 16, wherein each pair of nucleic acid probes is specific to each polynucleotide analyte; and wherein each pair of nucleic acid probes is configured to hybridize to a unique bridge probe in the presence of the polynucleotide analyte.
19. A method of detecting two or more polynucleotide analytes in a sample, the method comprising:
 - a) contacting a sample with a library according to claim 18, and
 - b) detecting each polynucleotide analyte based on hybridization to a unique bridge probe in the presence of the polynucleotide analyte.

20. The method of claim 19, wherein the method comprises contacting the sample with a unique bridge probe for each polynucleotide analyte.
21. The method of claim 20, wherein the unique bridge probe comprises a specific tag or barcode sequence.
22. The method of any one of claims 19 to 21, wherein the two or more polynucleotide analytes are detected concurrently based on hybridization to a unique bridge probe for each polynucleotide analyte.
23. The method of any one of claims 19 to 22, wherein the two or more polynucleotide analytes are detected sequentially based on multiple rounds of hybridization to a unique bridge probe for each polynucleotide analyte.
24. The method of any one of claims 19 to 23, wherein the method comprises detecting the unique bridge probe via hybridization to a readout probe that is conjugated to a label.
25. The method of claim 24, wherein the method comprises contacting the sample with a unique readout probe for each polynucleotide analyte.
26. The method of any one of claims 19 to 25, wherein the method comprises removing any bound or unbound bridge and/or readout probe in between detection of each polynucleotide analyte.
27. The method of any one of claims 19 to 26, wherein the method comprises removing any signal from any bound or unbound readout probe in between detection of each polynucleotide analyte.
28. A method of detecting or visualising the expression of one or more polynucleotide analytes in a sample, the method comprising a) contacting a sample with a library according to claim 18, and b) detecting or visualising the one or more polynucleotide analytes based on hybridisation to a unique bridge probe in the presence of the one or more polynucleotide analytes.

29. A kit comprising a pair of non-naturally occurring nucleic acid probes according to any one of claims 1 to 14 or a plurality of probe systems according to claims 15 or 16 or a library according to claim 18.
30. The kit of claim 29, wherein the kit further comprises one or more bridge probes.