Claims

What is claimed is:

1. A method of identifying a binding partner of at least one target molecule within a plurality of analyte molecules, wherein the target molecule is physically combined with a target labeling nucleic acid molecule, the target labeling nucleic acid molecule comprising a specific nucleotide sequence suitable for identifying the target molecule combined therewith, the method comprising:
   - contacting the target molecule with the plurality of analyte molecules, thereby forming a mixture,
     wherein the binding partner is suspected to be a member of the plurality of analyte molecules, and wherein each member of the plurality of analyte molecules is physically combined with an analyte labeling nucleic acid molecule, each analyte labeling nucleic acid molecule comprising a selected nucleotide sequence suitable for identifying the analyte molecule combined therewith;
   - allowing the formation of a complex between the target molecule and the binding partner thereof within the mixture;
   - subdividing the mixture into a plurality of compartments, such that each compartment comprises at most about one member of the group consisting of:
     (i) one target molecule, and
     (ii) one complex between a target molecule and an analyte molecule;
     thereby segregating a complex between a target molecule and an analyte molecule from the residual members of the plurality of analyte molecules;
   - allowing the target labeling nucleic acid molecule and the analyte labeling nucleic acid molecule to be linked,
     thereby forming a composite nucleic acid molecule;
   - allowing the plurality of compartments to disintegrate;
   - retrieving the composite nucleic acid molecule; and
   - determining the sequence of the analyte labeling nucleic acid molecule, thereby identifying the binding partner of the target molecule.

2. The method of claim 1, wherein retrieving the composite nucleic acid molecule

comprises at least one of:

(i) carrying out a primer based nucleic acid amplification using at least one primer that is complementary to a part of the sequence of the target labeling nucleic acid molecule,

thereby amplifying the composite nucleic acid molecule; and

(ii) • adding to the mixture a capture probe, the capture probe being capable of associating to the complex between the target molecule and the binding partner thereof, and

• retrieving the capture probe, thereby retrieving the complex between the target molecule and the binding partner thereof.

3. The method of claim 1 ~~or 2~~, wherein subdividing the mixture into a plurality of compartments is carried out such that each compartment comprises at most about one member of the group consisting of:

(i) one target molecule,

(ii) one complex between a target molecule and an analyte molecule, and

(iii) one analyte molecule.

~~4. The method of any one of claims 1 – 3, wherein the physical combination between the analyte molecules, suspected to be capable of forming a complex with the target molecule, and the analyte labelling nucleic acid molecules combined therewith comprises one of a covalent bond, a non-covalent bond, a linking molecule, a cell, a virus, a phage and a ribosome.~~

5. 4. The method of ~~any one of~~ claims 1 ~~–~~4, further comprising:

after subdividing the mixture into compartments releasing the physical combination between the analyte molecule defining the binding partner of the target molecule and the analyte labeling nucleic acid molecule combined therewith, thereby releasing the analyte labeling nucleic acid molecule.

6. 5. A method of identifying a pair of a first and a second binding partner within a plurality of peptides and/or proteins, wherein the first and the second binding partners are capable of forming a complex, the method comprising:

- providing a plurality of peptides and/or proteins, in a plurality of cells, wherein each member of the plurality of peptides and/ or proteins is physically combined with a member encoding nucleic acid molecule, the member encoding nucleic acid molecule comprising a nucleotide sequence encoding the peptide or protein combined therewith;
- subdividing the plurality of peptides and/or proteins in a plurality of cells, into compartments, such that each compartment comprises at most about one member or about one complex between members of the plurality of peptides and/ or proteins, in a plurality of cells,
  thereby segregating a complex between the first and the second binding partner from the residual peptides and/ or proteins;
- allowing the member encoding nucleic acid molecule of the first binding partner and the member encoding nucleic acid molecule of the second binding partner to be linked,
  thereby forming a composite nucleic acid molecule;
- allowing the compartments to disintegrate;
- retrieving the composite nucleic acid molecule; and
- determining the sequence of the composite nucleic acid molecule, thereby identifying the first and/or the second binding partner.

7. 6. The method of claim ~~6~~ 5, wherein retrieving the composite nucleic acid molecule comprises:
   - carrying out a primer based nucleic acid amplification using a primer that is complementary to a part of the sequence of the member encoding nucleic acid molecule,
     thereby amplifying the composite nucleic acid molecule.

~~8. The method of claim 7, wherein the primer based nucleic acid amplification is one of a polymerase chain reaction (PCR) and isothermal amplification.~~

~~9. The method of claim 8, wherein the isothermal amplification is one of strand displacement amplification (SDA), helicase based amplification and rolling circle amplification.~~

~~10. The method of claim 8, wherein the PCR is carried out using a pair of a first and a second primer, wherein the first primer is complementary to a part of the sequence of the target labeling nucleic acid molecule, and the second primer is a universal primer.~~

~~11. The method of any one of claims 6 - 10, wherein the plurality of peptides and/or proteins is formed by contacting the first binding partner with a plurality of peptides and/or proteins suspected to comprise the second binding partner.~~

~~12. The method of claim 55, wherein the first binding partner is a member of a first plurality of peptides and/or proteins and the second binding partner is suspected to be a member of a second plurality of peptides and/or proteins, the first plurality of peptides and/or proteins being combined with the second plurality of peptides and/or proteins, thereby forming said plurality of peptides and/or proteins.~~

13. 7. The method of ~~any one of~~ claims ~~6~~ 5 ~~12~~, wherein the composite nucleic acid molecule formed by a linkage of the member encoding nucleic acid molecule of the first binding partner and the member encoding nucleic acid molecule of the second binding partner is ~~physically~~ combined with ~~the complex between~~ the complex between the first and the second binding partner.

14. 8. The method of ~~any one of~~ claims ~~6~~ 5 ~~13~~, further comprising:
after subdividing the mixture into compartments releasing the physical combination between the member encoding nucleic acid molecules and the binding partners.

~~15. The method of any one of claims 6 - 14, wherein the physical combination between the members of the plurality of peptides and/or proteins and the member encoding nucleic acid molecules comprises one of a covalent bond, a non-covalent bond, a linking molecule, a cell, a virus, a phage and a ribosome.~~

16. 9. The method of claim ~~15~~ 5, wherein the physical combination is a cell and wherein each member of the plurality of peptides and/or proteins is covalently linked to one of a first and a second complementing moiety,

wherein the first and the second complementing moiety, when brought into physical proximity, complement each other, thereby together defining a reporter factor.

~~17. The method of claim 16, wherein the reporter factor is a factor that is capable of activating the expression of a protein affecting the phenotype of a suitable host cell.~~

~~18. The method of claim 17, wherein the first and the second complementing moiety are two fragments of the protein.~~

~~19. The method of claim 18, wherein the two fragments define two domains of the protein.~~

~~20. The method of claims 16 to 19, wherein the first and the second complementing moiety are capable of being expressed in the cell.~~

21. 10. The method of claim ~~20~~ 9, wherein each member of the plurality of peptides and/ or proteins and the complementing moiety are comprised in a fusion protein encoded by the member encoding nucleic acid molecule.

22. 11. The method of claim ~~21~~10, wherein the plurality of peptides and/ or proteins is provided by expressing under suitable conditions a plurality of member encoding nucleic acid molecules in a plurality of cells, wherein each member encoding nucleic acid molecule encodes a single fusion protein, each fusion protein comprising one member of the plurality of peptides and/ or proteins and one of the first and the second complementing moiety.

23. 12. The method of claim ~~22~~11, comprising allowing the expression of only one pair of a first and a second member encoding nucleic acid molecules in each of the plurality of cells, wherein the first nucleic acid molecule encodes a fusion protein having the first complementing moiety and the second nucleic acid molecule encodes a fusion protein having the second complementing moiety.

~~24. The method of claim 23, wherein the plurality of peptides and/ or proteins is expressed using a two hybrid system.~~

~~25. The method of any one of claims 6 – 24, further comprising purifying the amplified composite nucleic acid molecule.~~

~~26. The method of claims 6 - 25, wherein the plurality of peptides and/ or proteins is provided in a liquid suitable for handling nucleic acid molecules and peptides and/or proteins.~~

27. 13. The method of ~~any one of~~ claims ~~6-5~~ ~~–26~~, wherein the member encoding nucleic acid molecule of the first binding partner and the member encoding nucleic acid molecule of the second binding partner are allowed to be linked after the mixture is divided into compartments.

28. 14. The method of claim 13 ~~27~~, wherein the member encoding nucleic acid molecule of the first binding partner and the member encoding nucleic acid molecule of the second binding partner are allowed to be linked within a compartment obtained by subdividing the mixture into compartments.

29. 15. The method of ~~any one of~~ claims ~~6-5 – 28~~, wherein allowing the member encoding nucleic acid molecules to be linked comprises ligation of the nucleic acid molecules or overlap extension polymerase chain reaction.

~~30. The method of any one claims 6 - 29, further comprising:~~

~~allowing the formation of a complex between the first and the second binding partner within the plurality of peptides.~~

31. 16. A method of identifying a binding partner of a target peptide or protein within a plurality of peptides and/or proteins, wherein the target peptide or protein is comprised in a member of a first plurality of peptides and/ or proteins and the binding partner is suspected to be comprised in a member of a second plurality of peptides and/ or proteins, wherein each member of the first and the second plurality of peptides and/ or proteins is physically combined with a member encoding nucleic acid molecule, the member encoding nucleic acid molecule comprising a nucleotide sequence encoding the peptide or protein combined therewith,

the method comprising:

- combining the first and the second plurality of peptides and/ or proteins, thereby forming a mixture;
- allowing the formation of a complex between the target peptide or protein and the binding partner;
- subdividing the mixture into compartments, such that each compartment comprises at most about one member or about one complex between members of the combined pluralities of peptides and/ or proteins;
- allowing the member encoding nucleic acid molecule of the target peptide or protein and the member encoding nucleic acid molecule of the binding partner of the formed complex to be linked,
  thereby forming a composite nucleic acid molecule;
- allowing the compartments to disintegrate;
- retrieving the composite nucleic acid molecule; and
- determining the sequence of the composite nucleic acid molecule, thereby identifying the binding partner.

32. 17. The method of claim ~~31~~ 16, wherein retrieving the composite nucleic acid molecule comprises one of:

(i) carrying out a primer based nucleic acid amplification using a primer that is complementary to a part of the sequence of the member encoding nucleic acid molecule encoding the target peptide or protein,
thereby amplifying the composite nucleic acid molecule; and

(ii) • adding to the mixture a capture probe, the capture probe being capable of associating to the complex between the target peptide or protein and the binding partner thereof, and
• retrieving the capture probe, thereby retrieving the complex between the target peptide or protein and the binding partner thereof.

~~33. The method of claim 32, wherein the primer based nucleic acid amplification is one of a polymerase chain reaction (PCR) and isothermal amplification.~~

~~34. The method of claim 33, wherein the isothermal amplification is one of strand displacement amplification (SDA), helicase based amplification and rolling circle amplification.~~

~~35. The method of claim 33, wherein the PCR is carried out using a pair of a first and a second primer, wherein the first primer is first primer is complementary to a part of the sequence of the member encoding nucleic acid molecule encoding the target peptide or protein, and the second primer is a universal primer.~~

~~36. The method of any one of claims 31 35, further comprising purifying the amplified composite nucleic acid molecule.~~

~~37. The method of claim 36, wherein purifying the amplified composite nucleic acid molecule comprises extracting the same.~~

~~38. The method of any one of claims 31 37, wherein the physical combination between the members of the first plurality of peptides and/or proteins and the member encoding nucleic acid molecules comprises one of a covalent bond, a non covalent bond, a linking molecule, a cell, a virus, a phage and a ribosome.~~

~~39. The method of any one of claims 31 38, wherein the physical combination between the members of the second plurality of peptides and/or proteins and the member encoding nucleic acid molecules comprises one of a covalent bond, a non covalent bond, a linking molecule, a cell, a virus, a phage and a ribosome.~~

40. 18. The method of claim ~~39~~ 16, wherein the physical combination between the members of the first and of the second plurality of peptides and/or proteins is a cell and wherein each member of the first plurality of peptides and/or proteins is covalently linked to a first complementing moiety and each member of the second plurality of peptides and/or proteins is covalently linked to a second complementing moiety,

wherein the first and the second complementing moiety, when brought into physical proximity, complement each other, thereby together defining a reporter factor.

~~41. The method of claim 40, wherein the reporter factor is a factor that is capable of activating the expression of a protein affecting the phenotype of a suitable host cell.~~

~~42. The method of claims 40 or 41, wherein the first and the second complementing moiety are two fragments of the protein.~~

~~43. The method of claim 42, wherein the two fragments are two domains of the protein.~~

~~44. The method of claims 40 to 43, wherein the first and the second complementing moiety are capable of being expressed in the cell.~~

45. 19. The method of claim ~~44~~ 16, wherein each member of the first plurality of peptides and/or proteins and the first complementing moiety are comprised in a fusion protein encoded by the member encoding nucleic acid molecule of the member of the first plurality of peptides and/or proteins.

46. 20. The method of claims ~~44 or 45~~ 16, wherein each member of the second plurality of peptides and/or proteins and the second complementing moiety are comprised in a fusion protein encoded by the member encoding nucleic acid molecule of the member of the second plurality of peptides and/or proteins.

47. 21. The method of claim ~~46~~ 20, wherein each member of the first plurality of peptides and/or proteins and the first complementing moiety are comprised in a fusion protein encoded by the member encoding nucleic acid molecule of the member of the first plurality of peptides and/or proteins, and each member of the second plurality of peptides and/or proteins and the second complementing moiety are comprised in a fusion protein encoded by the member encoding nucleic acid molecule of the member of the second plurality of peptides and/or proteins,

wherein said fusion proteins are provided by expressing under suitable conditions a plurality of member encoding nucleic acid molecules of the first plurality of peptides and/or proteins and a plurality of member encoding nucleic acid molecules of the second plurality of peptides and/or proteins.

48. 22. The method of claim ~~47~~ 21, comprising allowing the expression of only one pair of about one member encoding nucleic acid molecule of the first plurality of peptides and/or proteins and about one member encoding nucleic acid molecule

of the second plurality of peptides and/or proteins in each of the plurality of cells.

49. 23. A method of identifying one or more pairs of a first and a second binding partner within a plurality of peptides and/or proteins, wherein the first and the second binding partner are capable of forming a complex, the method comprising:

- providing a library of nucleic acid molecules encoding a plurality of peptides and/or proteins, wherein the plurality of peptides and/ or proteins is suspected to comprise the one or more pairs of a first and a second binding partner;
- providing a plurality of members of a first vector and a plurality of members of a second vector, the first vector having a nucleic acid sequence encoding a first complementing moiety, the second vector having a nucleic acid sequence encoding a second complementing moiety, wherein the first and the second complementing moiety, when brought into physical proximity, complement each other, thereby together defining a reporter factor,
- providing each member of the two pluralities of a first and of a second vector with one nucleic acid molecule of the library of nucleic acid molecules encoding the plurality of peptides and/or proteins,
- introducing one of the members of the first vector and one of the members of the second vector into the same suitable cell, wherein both the first and the second vector each have one nucleic acid molecule encoding one of the plurality of peptides and/or proteins,
- allowing in the cell the expression of the pair of peptides and/or proteins encoded by the nucleic acids provided with the vectors,
- collecting any cell in which the formation of the reporter factor is detected,
- subdividing individual collected cells in which the formation of the reporter factor is detected into compartments, such that each compartment comprises at most about one cell,
- allowing in the compartments the member encoding nucleic acid molecule of the first binding partner and the member encoding nucleic acid molecule of the second binding partner to be linked,

thereby forming a composite nucleic acid molecule;

- allowing the compartments to disintegrate;
- retrieving the composite nucleic acid molecule; and
- determining the sequence of the composite nucleic acid molecule, thereby identifying the first and/or the second binding partner.

50. 24. The method of claim ~~49~~ 23, wherein the first vector is a plasmid encoding the first complementing moiety, wherein the plurality of members of the first vector is a plurality of molecules of the first plasmid and wherein providing each member of the first vector with one nucleic acid molecule is inserting one nucleic acid molecule into each member of the pluralities of molecules of the first plasmid.

51. 25. The method of claims ~~49 or 50~~ 23, wherein the second vector is a plasmid encoding the second complementing moiety, wherein the plurality of members of the second vector is a plurality of molecules of the second plasmid and wherein providing each member of the second vector with one nucleic acid molecule is inserting one nucleic acid molecule into each member of the pluralities of molecules of the second plasmid.

~~**52.** The method of any one of claims 49 – 51, wherein the reporter factor is a factor that is capable of activating the expression of a protein affecting the phenotype of the suitable cell.~~

~~**53.** The method of claim 49, wherein the first and the second complementing moiety are two fragments of the protein.~~

~~**54.** The method of claim 53, wherein the two fragments are two domains of the protein.~~

55. 26. The method of ~~any one of~~ claims ~~49 – 54~~ 23, wherein retrieving the composite nucleic acid molecule comprises at least one of:

(i) carrying out a primer based nucleic acid amplification using a primer that is complementary to a part of the sequence of the member encoding nucleic acid molecule of the first binding partner,
thereby amplifying the composite nucleic acid molecule; and

(ii) • adding to the mixture a capture probe, the capture probe being capable of associating to the complex between the first and a second binding partner, and

• retrieving the capture probe, thereby retrieving the complex between the first and a second binding partner.

~~56. The method of claim 55, wherein the primer based nucleic acid amplification is one of a polymerase chain reaction (PCR) and isothermal amplification.~~

~~57. The method of claim 56, wherein the isothermal amplification is one of strand displacement amplification (SDA), helicase based amplification and rolling circle amplification.~~

~~58. The method of claim 56, wherein the PCR is carried out using a pair of a first and a second primer, wherein the first primer is complementary to a part of the sequence of the target labeling nucleic acid molecule, and the second primer is a universal primer.~~

59. 27. A method of identifying a binding partner of at least one target peptide or protein within a plurality of analyte peptides and/or proteins, wherein the target peptide or protein and the binding partner thereof are capable of forming a complex, the method comprising:

- providing a library of nucleic acid molecules encoding the plurality of analyte peptides and/or proteins, wherein the plurality of peptides and/ or proteins is suspected to comprise a binding partner of the at least one target peptide or protein;
- providing at least one nucleic acid molecule encoding the at least one target peptide or protein;
- providing a plurality of members of a first vector and a plurality of members of a second vector, the first vector having a nucleic acid sequence encoding a first complementing moiety, the second vector having a nucleic acid sequence encoding a second complementing moiety, wherein the first and the second complementing moiety, when brought into physical proximity, complement each other, thereby together defining a reporter factor,

- providing each member of the plurality of members of the first vector with one nucleic acid molecule of the library of nucleic acid molecules encoding the plurality of analyte peptides and/or proteins,
- providing each member of the plurality of molecules of the second vector with one nucleic acid molecule of the at least one nucleic acid molecule encoding the at least one target peptide or protein,
- introducing one of the members of the first vector and one of the members of the second vector into the same suitable cell,
- allowing in the cell the expression of the pair of peptides and/or proteins encoded by the nucleic acids provided with the vectors,
- collecting any cell in which the formation of the reporter factor is detected,
- subdividing individual collected cells in which the formation of the reporter factor is detected into compartments, such that each compartment comprises at most about one cell,
- allowing in the compartments the member encoding nucleic acid molecule of the first binding partner and the member encoding nucleic acid molecule of the second binding partner to be linked,
  thereby forming a composite nucleic acid molecule;
- allowing the compartments to disintegrate;
- retrieving the composite nucleic acid molecule; and
- determining the sequence of the composite nucleic acid molecule, thereby identifying the binding partner of the target peptide or protein.

~~60. The method of claim 59, wherein the reporter factor is a factor that is capable of activating the expression of a protein affecting the phenotype of the suitable cell.~~

61. 28. The method of claims ~~59 or 60~~ 27, wherein the first vector is a plasmid encoding the first complementing moiety, wherein the plurality of members of the first vector is a plurality of molecules of the first plasmid and wherein providing each member of the first vector with one nucleic acid molecule is inserting one nucleic acid molecule into each member of the pluralities of molecules of the first plasmid.

62. 29. The method of ~~any one of~~ claims ~~59 or 61~~ 27, wherein the second vector is a plasmid encoding the second complementing moiety, wherein the plurality of

members of the second vector is a plurality of molecules of the second plasmid and wherein providing each member of the second vector with one nucleic acid molecule is inserting one nucleic acid molecule into each member of the pluralities of molecules of the second plasmid.

63. 30. The method ~~of one~~ of claims ~~59 62~~ 27, wherein retrieving the composite nucleic acid molecule comprises at least one of:

(i) carrying out a primer based nucleic acid amplification using a primer that is complementary to a part of the sequence of the nucleic acid molecule encoding the target peptide or protein,

thereby amplifying the composite nucleic acid molecule; and

(ii) • adding to the mixture a capture probe, the capture probe being capable of associating to the complex between the target peptide or protein and the binding partner thereof, and

• retrieving the capture probe, thereby retrieving the complex between the target peptide or protein and the binding partner thereof.

~~**64.** The method of claim63, wherein the primer based nucleic acid amplification is one of a polymerase chain reaction (PCR) and isothermal amplification.~~

~~**65.** The method of claim 64, wherein the isothermal amplification is one of strand displacement amplification (SDA), helicase based amplification and rolling circle amplification.~~

~~**66.** The method of claim 64, wherein the PCR is carried out using a pair of a first and a second primer, wherein the first primer is complementary to a part of the sequence of the nucleic acid molecule encoding the target peptide or protein, and the second primer is a universal primer.~~

67. 31. The method of ~~any one of~~ claims ~~59 66~~ 27, being a method of identifying a binding partner of at least two target molecules within a plurality of analyte peptides and/or proteins, wherein providing at least one nucleic acid molecule encoding the at least one target peptide or protein is carried out by providing at least two nucleic acid molecules, each nucleic acid molecule encoding one of the at least two target peptides or proteins.

~~**68.** A kit of parts for identifying a binding partner of a target peptide or protein, the kit comprising:~~

- ~~a plurality of composite nucleic acid molecules, wherein each composite nucleic acid molecule comprises the sequences of a pair of a first and a second peptide and/or protein, the first and the second peptide and/or protein defining binding partners capable of forming a complex with each other~~
- ~~a universal primer.~~

~~**69.** The kit of claims 68, wherein the plurality of composite nucleic acid molecules is a library of at least 10^3, of at least 10^4, of at least 10^5 or of at least 10^6 nucleic acid molecules.~~

~~**70.** The kit of claims 68 or 69, wherein the plurality of composite nucleic acid molecules is obtainable by carrying out the method of any one of claims 1, 48, 80, 101 and 111.~~