

CLAIMS

1. A multicomponent system wherein

a first component is an engineered TCR-presenting cell (eTPC), designated component A, wherein component A lacks endogenous surface expression of at least one family of analyte antigen-presenting complexes (aAPX) and/or analyte antigenic molecule (aAM), lacks endogenous expression of TCR chains alpha, beta, delta and gamma, and expresses CD3 proteins and contains two further single components designated B and D, which are different and each respectively a genomic receiver site for integration of a single ORF encoding at least one analyte TCR chain of alpha, beta, delta or gamma, and/or two ORFs encoding a pair of analyte TCR chains, and wherein the genomic receiver sites B and D are each selected from

a. A synthetic construct designed for recombinase mediated cassette exchange (RMCE)

b. A synthetic construct designed for site directed homologous recombination;

and

a second component is a genetic donor vector, **designated component C**, for delivery of ORF encoding analyte TCR chains, wherein **component C**, is matched to component **B**, and wherein the component **C** is designed to deliver

c. A single ORF encoding at least one analyte TCR chain of alpha, beta, delta and/or gamma and/or

d. Two ORFs encoding a pair of analyte TCR chains,

and wherein c and/or d optionally encodes a selection marker of integration, such that the analyte TCR chains can be expressed as TCR surface protein in complex with the CD3 (**TCRsp**) on component **A**.

2. A multicomponent system according to **claim 1** wherein the family of **aAPX** may be any of the following

a. HLA class I

- b. HLA class II
 - c. non-HLA antigen-presenting complex.
- 3. A multicomponent system according to **claims 1 or 2**, wherein a further component **designated E** is a genetic vector matched to **D**, wherein component **E** is designed to deliver a single ORF encoding one analyte TCR chain of alpha, beta, delta or gamma, such that when a ORF encoding the complementary paired analyte TCR chain of alpha, beta, delta or gamma is integrated into component **B**, expressed as **TCRsp** on component **A** and optionally wherein component **E** encodes a selection marker of integration.
- 4. A multicomponent system according to **any of the preceding claims**, wherein component **A**, further contains a component **designated F**, a synthetic genomic TCR-stimulation response element selected from

 - a. A single component synthetic construct containing at least one native promoter and/or at least one synthetic promoter and at least one reporter
 - b. A multi-component synthetic construct designed with at least one native promoter and/or at least one synthetic promoter and at least one reporter,

and wherein activation of a and/or b is dependent on at least one signal transduction pathway selected from a synthetic pathway, a native pathway or a combination thereof.
- 5. A multicomponent system according to claim 1, wherein the genomic receiver sites B and D are included and are both a synthetic construct designed for recombinase mediated cassette exchange (RMCE).
- 6. A multicomponent system according to any of the preceding **claims** wherein the **aAM** is selected from

 - a. a polypeptide or complex of polypeptides translated from the analyte antigenic molecule ORF(s)

- b. a peptide derived from a polypeptide translated from the analyte antigenic molecule ORF(s)
 - c. a peptide derived from altering the component A proteome
 - d. a polypeptide derived from altering the component A proteome
- 5
 - e. a metabolite derived from altering the component A metabolome.
- 7. A multicomponent system according to any of **the preceding claims** wherein component **A** expresses CD4 and/or CD8.
- 8. A multicomponent system according to any of **the preceding claims** wherein component **A** expresses additional TCR co-receptors.
- 10
 - 9. A multicomponent system according to **claim 8** wherein component **A** expresses CD28 and/or CD45.
 - 10. A multicomponent system according to **any of claims 2-9** wherein the **component B and/or D** is included and comprises of at least one of the following genetic elements
- 15
 - a. Heterospecific recombinase sites
 - b. Homologous arms
 - c. Eukaryotic promoter
 - d. Eukaryotic conditional regulatory element
 - e. Eukaryotic terminator
- 20
 - f. Selection marker
 - g. Splice acceptor site
 - h. Splice donor site
 - i. Non-protein coding gene
 - j. Insulator

- k. Mobile genetic element
- l. Meganuclease recognition site
- m. Internal ribosome entry site (IRES)
- n. Viral self-cleaving peptide element,
- 5 o. A Kozak consensus sequence.

11. A multicomponent system according to any of **preceding claims** wherein the **component C and/or E** is included and comprises of at least one of the following genetic elements

- a. Heterospecific recombinase sites
- 10 b. Homologous arms
- c. Eukaryotic promoter
- d. Eukaryotic conditional regulatory element
- e. Eukaryotic terminator
- f. Selection marker
- 15 g. Selection marker of integration
- h. Splice acceptor site
- i. Splice donor site
- j. Non-protein coding gene
- k. Insulator
- 20 l. Mobile genetic element
- m. Meganuclease recognition site
- n. Internal ribosome entry site (IRES)
- o. A viral self-cleaving peptide element

- p. An antibiotic resistance cassette
- q. A bacterial origin of replication
- r. A yeast origin of replication
- s. A cloning site
- 5 t. A kozak consensus sequence.

12. A multicomponent system according to **any of claims 1-13**, wherein the component **B** and/or **D** is included and is for RMCE integration of a single ORF and comprises:

- a. A Eukaryotic promoter
- 10 b. A pair of heterospecific recombinase sites
- c. A Kozak consensus sequence
- d. A selection marker
- e. A Eukaryotic terminator.

13. A multicomponent system according to **any of the preceding claims** wherein component **C and/or E** is present is for RMCE integration of a single ORF and comprises the following genetic elements:

- a. A pair of heterospecific recombinase sites
- b. A Kozak consensus sequence
- c. An antibiotic resistance cassette
- 20 d. A bacterial of replication
- e. A cloning site for introduction of a single ORF encoding one or more TCR chains and/or selection marker of integration.

14. A multicomponent system according to **any of the preceding claims** wherein component C and/or E is included and is combined with at least one ORF encoding at least one analyte TCR chain to obtain component C'

25

and/or E.'

15. A multicomponent system according to **claim 14** wherein the combination is performed multiple times to obtain a library of component C' and/or E'.
- 5 16. A multicomponent system according to claim 14 **or 15**, wherein the analyte TCR chain encoding sequences are derived from
 - a. Paired cloning of TCR chain ORF sequence(s) from primary T-cells
 - b. Unpaired cloning of TCR chain ORF sequence(s) from primary T-cells
 - c. Synthetic TCR chain ORF sequence(s).
- 10 17. A multicomponent system according to any of **claims 14-16**, wherein one or more component C' and/or E' is combined with component A, to integrate two complementary analyte TCR chains encoded in component C' and/or E', into components **B** and/or **D**, to obtain a cell, **designated an eTPC-t**, wherein components B and/or D become components B' and/or D' such that

15 it expresses a **TCRsp** on the surface of component A.
- 20 18. A multicomponent system according to any of **claims 14-17**, wherein one of component C' or E' is combined with component A, to integrate one analyte TCR chain encoded in component C' or E', into components **B** or **D**, to obtain a cell, **designated eTPC-x**, wherein components B or D become components B' or D' such that the eTPC-x expresses a single TCR chain.
- 25 19. A multicomponent system according to any of **claims 15-18**, wherein one of component C' or E' is combined with an **eTPC-x**, to integrate one analyte TCR chain encoded in component C' or E' that is complementary to the TCR chain expressed in the **eTPC-x**, into components **B** or **D**, of the **eTPC-x**, to obtain an **eTPC-t**, wherein components B or D become components B' or D' such that it expresses an **TCRsp** on the surface of the eTPC-t.
20. A method for preparing an eTPC-t as defined in **claim 17**, the method comprising
 - a. Combining component A, with at least one of component C' and/or

E', wherein the one or more component C' and/or E' encode one or more pairs of complementary analyte TCR chains, and combining with integration factors and at least one of

- b. Selecting for loss of genomic receiver site selection marker(s)
- 5 c. Selecting for gain of a surface expression of the **TCRsp** and/or one or more **CD3** proteins
- d. Selecting for gain of one or more of a selection marker of integration.

21. A method according to **claim 20**, wherein b, c and d are included.

10 22. A method according to **claim 20 or 21**, wherein the one or more component C' and/or E' encodes a single complementary pair of TCR chains in step a.

23. A method according to **claim 22**, wherein the method is conducted multiple times wherein each time step a is performed using a distinct single complementary pair of TCR chains, such that a distinct **eTPC-t** is obtained,
15 to obtain a library of discrete and defined **eTPC-t**.

24. A method according to **claim 22-23** wherein the one or more component C' and/or E' encodes a mixed pool of two or more distinct complementary pair of TCR chains in step a of claim 22, to obtain a library, wherein the library is comprised of a mixed population of **eTPC-t**, wherein each **eTPC-t**
20 expresses a single **TCRsp** derived from the pool used in step a of claim 23.

25. A method for preparing an **eTPC-x** as defined in **claim 18** the method comprising

- a. Combining component A, with one or more of component C' or one or more component E', wherein one or more component C' or one or more E' encodes an analyte TCR chain, and combining with integration factors and at least one of
- 25 b. Selecting for loss of genomic receiver site selection marker(s)
- c. Selecting for gain of one or more of a selection marker of integration.

26. A method according to **claim 25** wherein b and c are included.
27. A method according to **claim 25 or 26**, wherein one component C' or one component E' encodes a single analyte TCR chain in step a.
- 5 28. A method according to **claim 25** wherein the method is conducted multiple times wherein each time step a is performed using a distinct analyte TCR chain, such that a distinct **eTPC-x** is obtained, to obtain a library of discrete and defined **eTPC-x**.
- 10 29. A method according to **claim 25 or 26**, wherein one or more component C' or one or more component E' encodes a mixed pool of two or more single analyte TCR chains in step a, to obtain a library, wherein the library is comprised of a mixed population of **eTPC-x**, wherein each **eTPC-x** expresses a single **analyte TCR chain** derived from the pool used in step a.
30. A method for preparing an **eTPC-t** as defined in **claim 18** the method comprising
- 15 a. Combining an **eTPC-x**, with one or more of component C' or one or more component E', wherein one or more component C' or one or more E' encodes an analyte TCR chain that is complementary to the integrated analyte TCR chain in the **eTPC-x**, and combining with integration factors and at least one of
- 20 b. Selecting for loss of genomic receiver site selection marker(s)
- c. Selecting for gain of a surface expression of the **TCRsp** and/or one or more **CD3** proteins
- d. Selecting for gain of one or more of a selection marker of integration.
- 25 31. A method according to **claim 30**, wherein b, c and d are included.
32. A method according to **claim 30 or 31**, wherein one component C' or one component E' encodes a single analyte TCR chain in step a.
33. A method according to **claim 31**, wherein the method is conducted multiple times, wherein each time step a is performed using a distinct analyte TCR

chain, such that a distinct **eTPC-t** is obtained expressing a **TCRsp**, to obtain a library of discrete and defined **eTPC-t**.

5 34. A method according to **claim 30-31** wherein one or more component C' or one or more component E' encodes a mixed pool of two or more single analyte TCR chains in step a, to obtain a library, wherein the library is comprised of a mixed population of **eTPC-t**, wherein each **eTPC-t** expresses a single **TCRsp** derived from the pool used in step a.

35. An eTPC-t obtained from the multicomponent system according to any of **claims 1-19** for use in an eTPC:A analytical device for characterisation of

- 10 a. specificity of the expressed analyte **TCRsp** to an **analyte antigen** and/or
- b. affinity of the expressed analyte **TCRsp** to an **analyte antigen** and/or
- 15 c. a signal response of the expressed analyte **TCRsp** to an **analyte antigen**

wherein an analyte antigen is selected from an **aAPX:aAM** and/or **aAM** and/or **aAPX** and/or **aAPX:CM** and/or **affinity reagent**, wherein the analyte antigen is in the form of at least of the following, a soluble reagent, an immobilised reagent, presented by a non-cell based particle (**NCBP**),

20 presented on the surface of a cell (**analyte APC**), wherein **aAPX:CM** is an **aAPX** that is loaded with a cargo molecule (**CM**), and wherein **aAPX:aAM** is an **aAPX** is loaded with an **aAM** as **CM**.

36. A multicomponent system according to any of claims 1-19 for use in generating at least one analyte eTPC-t.

25 37. An analyte eTPC characterized in that it

- a. Contains 2 genomic receiver sites, designated B and D, each of which may comprise a single ORF encoding at least one analyte TCR chain of alpha, beta, delta or gamma, and/or two ORFs encoding a pair of analyte TCR chains

b. Expresses CD3 proteins

c. Does not express any other TCR chains than those comprised in B and D,

and wherein the genomic receiver sites B and D are each selected from

5 a. A synthetic construct designed for recombinase mediated cassette exchange (RMCE)

b. A synthetic construct designed for site directed homologous recombination.

38. An analyte eTPC obtainable from the multicomponent system as defined in
10 any of claims 1–19 characterized in that it

a. Contains 2 genomic receiver sites, designated B and D, each of which may comprise a single ORF encoding at least one analyte TCR chain of alpha, beta, delta or gamma, and/or two ORFs encoding a pair of analyte TCR chains

15 b. Expresses CD3 proteins

c. Does not express any other TCR chains than those comprised in B and D,

and wherein the genomic receiver sites B and D are each selected from

20 a. A synthetic construct designed for recombinase mediated cassette exchange (RMCE)

b. A synthetic construct designed for site directed homologous recombination.