Claims

1. A combined system comprising two separate components, wherein a first component is a vector carrying variable and constant (V-C) T-cell receptor (TCR) gene segments, and a second component is a vector carrying joining (J) TCR gene segments,

wherein the first component is a V-C entry vector containing

a. origin of replication,
b. a first positive selection marker
c. 5' genetic element, or elements,
d. Kozak Sequence,
e. TCR variable gene segment,
f. a first Type IIS sequence, for site specific recognition and cleavage by a first Type IIS restriction enzyme, wherein the sequence is orientated such that the enzyme cleaves 5' of said recognition sequence and at the 3' end of the TCR variable gene segment,
g. a negative selection marker,
h. a second Type IIS sequence for site specific recognition and cleavage by a second Type IIS restriction enzyme, wherein the sequence is orientated such that the enzyme cleaves to direct cleavage 3' of said recognition sequence and at the 5' end of the TCR constant gene segment,.
i. TCR constant gene segment, and
j. 3' genetic element, or elements,

wherein the second component is a J donor vector containing

a. origin of replication,
b. a second positive selection marker,
c. a third Type IIS sequence for site specific recognition and cleavage by a third Type IIS restriction enzyme, wherein the sequence is orientated such that the enzyme cleaves 3' of said recognition sequence and at the 5' end of the TCR joining gene segment,
d. TCR Joining gene segment,
e. A C-part, corresponding to a small 5' portion of a constant gene segment, and
f. a fourth Type IIS sequence for site specific recognition and cleavage by fourth Type IIS restriction enzyme, wherein the sequence is orientated such that the enzyme cleaves 5‘ of said recognition sequence and at the

3' end TCR C-part portion of the construct, such that the single strand overhang sequence generated is complimentary with that generated by said second Type IIS sequence.

2. A combined system according to claim 1, wherein the 5' genetic element comprises one or more elements selected from

a. gene cis/acting element,
b. heterospecific recognition site for recombinase enzymes,
c. a 5' homologous recombination arm for a genomic site of interest,
d. a mRNA splice acceptor site,
e. an internal ribosomal entry site, and
f. an epigenetic insulator sequence.

3. A combined system according any of claims 1-2, wherein the negative selection marker is selected from

a. a restriction enzyme recognition site not contained elsewhere in the first component or within the TCR joining gene segment,
b. a bacterial suicide gene, and
c. a reporter element.

4. A combined system according to any of claims 1-3, wherein the 3' genetic element comprises one or more elements selected from

a. a terminator element,
b. heterospecific recognition site for recombinase enzymes,
c. a 3' homologous recombination arm for a genomic site of interest,
d. a mRNA splice donor site,
e. an internal ribosomal entry site, and
f. an epigenetic insulator sequence.

5. A combined system according to any of claims 1-4, wherein the components do not contain any Type IIS sequences further than those defined above, and only contain said negative selection marker.

6. A combined system according to any of the preceding claims further comprising a third component comprising an oligonucleotide duplex encoding CDR3 (odeCDR3), wherein the odeCDR3 has

a. a first single strand overhang sequence complimentary to that generated by cleavage of the first Type IIS restriction site at the 3' end of TCR variable gene segment,
b. a double strand segment encoding a TCR CDR3 region and devoid of negative selection marker, which negative selection marker is as defined in claim 5, and said double strand element is also devoid of any Type IIS restriction sequences of the first or second component, and
c. a second single strand overhang sequence complimentary to that generated by cleavage of the third Type IIS restriction enzyme site at the 5' end of TCR joining gene segment.

7. A combined system according to any of claims 1-5 further comprising a third component comprising an oligonucleotide duplex encoding CDR3 (odeCDR3), wherein the odeCDR3 is a dsDNA molecule or plasmid DNA encoding the CDR3 flanked by two Type IIS recognition and cleavage sites, such that cleavage by said enzymes results in single strand overhangs complimentary to those generated by the first Type IIS cleavage in the V-C entry vector at the 5' end, and with the third Type IIS cleavage of the J donor vector at the 3' end, to obtain a digested product of said odeCDR3 comprising a, b and c as described in claim 6.

8. A combined system according to any of claims 1-7, wherein the first to fourth Type IIS sequences are the same or different.

9. A two-part vector library comprising a library of a V-C entry vectors as defined in claim 1 and a library of J donor vectors as defined in claim 1, wherein the library of V-C entry vectors is

a collection of one or more vectors representing all germline TCR variable and constant gene segments of an organism having such TCRs, or

a collection of one or more vectors representing a collection of variant germline TCR variable and constant gene segments of an organism having such TCRs, such that translated amino acid sequence of the encoded protein is unmodified in relation to the protein sequence encoded by the germline gene segment, or

a collection of one or more vectors representing a collection of variant germline TCR variable and constant gene segments of an organism having such TCRs, such that translated amino acid sequence of the encoded protein is modified in relation to the protein sequence encoded by the germline gene segment and wherein

the J donor vector library is a collection of one or more vectors representing germline TCR joining gene segments of an organism having such TCRs, or

a collection of one or more vectors representing variant germline TCR joining gene segments of an organism having such TCRs, such that translated amino acid sequence of the encoded protein is unmodified in relation to the protein sequence encoded by the germline gene segment, or

a collection of one or more vectors representing variant germline TCR joining gene segments of an organism having such TCRs, such that translated amino acid sequence of the encoded protein is modified in relation to the protein sequence encoded by the germline gene segment.

10. A method for in vitro reconstitution of a full length TCR open reading frame (ORF), said method comprising

a. selecting a V-C entry vector,
b. selecting a J donor vector,
c. selecting an odeCDR3,
d. combining a, b and c to react with i) Type IIS restriction enzyme(s) to cleave all Type IIS restriction enzyme recognition and cleavage sites present in the V-C entry vector and in the J donor vector and ii) DNA ligase enzyme and subjecting the combined mix to a thermocycling reaction,
e. transforming the reaction product obtained from step d. into a host organism competent for DNA vector propagation, and
f. propagating said host organism to obtain full length reconstituted TCR open reading frame in the resulting V-C entry vector backbone.

11. A V-C entry vector selected from
sequence SEQ0692,

sequence SEQ0693, and
sequence SEQ0694.

12. A J donor vector represented by the sequence SEQ0700.

13. A library of V-C entry vectors as defined in any of claims 1-8 for use in combination with a library of J donor vectors and an odeCDR3 to reconstitute a full-length TCR ORF, wherein the V-C entry vectors contain Variable and Constant gene segments to recapitulate the gene segment usage of the human TRA locus, represented by the sequences SEQ0049 to SEQ0094; and/or the V-C entry vectors contain Variable and Constant gene segments to recapitulate the gene segment usage of the human TRB locus, represented by the sequences SEQ0484 to SEQ0577.

14. A library of J donor vectors as defined in any of claims 1-8, containing Joining gene segments to recapitulate the gene segment usage of the human TRA locus, represented by sequences SEQ0323 to SEQ0378, wherein the vectors are used in conjunction with an odeCDR3 spanning the entire CDR3 region; and/or
containing Joining gene segments to recapitulate the gene segment usage of the human TRA locus represented by sequences SEQ0379 to SEQ0434, wherein the vectors are used in conjunction with an odeCDR3 reduced in length by three to four codons.

15. A library of J donor vectors as defined in any of claims 1-8, containing Joining gene segments to recapitulate the gene segment usage of the human TRB locus, represented by sequences SEQ0636 to SEQ0661, wherein the vectors are used in conjunction with an odeCDR3 spanning the entire CDR3 region, and/or
containing Joining gene segments to recapitulate the gene segment usage of the human TRB locus, represented by sequences SEQ0662 to SEQ0687, wherein the vectors are used in conjunction with an odeCDR3 reduced in length by three to four codons.

16. A library according to claim 14 or 15 for use in combination with a V-C entry vector and an odeCDR3 to reconstitute a full-length TCR ORF.

17. A combined system according to any of claims 1-8, wherein said first component is a V-C entry vector encoding a first TCR chain, the system further comprises a component comprising a Bidirectional Terminator (BiT) donor vector, and a component comprising a V-C entry vector encoding a second TCR chain complimentary to said first TCR chain.

18. A combined system according to claim 17, wherein said V-C entry vector of said first componentfurther comprises

a. a fifth Type IIS sequence for site specific recognition and cleavage by a fifth Type IIS restriction enzyme, wherein the sequence is oriented such that the enzyme cleaves 5' of said recognition sequence, and at the 3' end of the constant gene segment,
b. a negative selection marker located 3' of the fifth Type IIS sequence,
c. a sixth Type IIS sequence for site specific recognition and cleavage by a sixth Type IIS restriction enzyme located 3' of the negative selection marker of b., wherein the sequence is oriented such that the enzyme cleaves 3' of said recognition sequence, and to the 5' of the 3' genetic element(s).

19. A combined system according to claim 17 or 18, wherein said V-C entry vector encoding a second TCR chain complimentary to said first chain and further comprises

a. a seventh Type IIS sequence for site specific recognition and cleavage by a seventh Type IIS restriction enzyme, wherein the sequence is oriented such that the enzyme cleaves 3' of said recognition sequence and at the 5' of the Kozak sequence,
b. an eighth Type IIS sequence for site specific recognition and cleavage by an eighth Type IIS restriction enzyme, wherein the sequence is oriented such that the enzyme cleaves 5' of said recognition sequence, and at the 3' end of the constant gene segment,
c. a negative selection marker located at the 3' of the eighth Type IIS sequence.

20. A combined system according to any of claims 17-19, wherein said BiT donor vector contains

an origin of replication,

a. a positive selection marker,
b. a ninth Type IIS sequence for site specific recognition and cleavage by a ninth Type IIS restriction enzyme, wherein the sequence is oriented such that the enzyme cleaves 3' of said recognition sequence and the 5' end of a bidirectional terminator element, d,
c. a bidirectional terminator element encoding two transcriptional terminators in antisense arrangement, and
d. a tenth Type IIS sequence for site specific recognition and cleavage by a tenth Type IIS restriction enzyme, wherein the sequence is oriented such that the enzyme cleaves 5' of said recognition sequence and the 3' of the bidirectional terminator element, d.

21. A combined system according to any of claims 17-20, wherein the first to fourth Type IIS sequences are the same or different.

22. A combined system according to any one of claims 17-20, wherein the fifth to tenth Type IIS sequences are the same or different, and are different from those of the first to fourth Type IIS sequences.

23. A combined system according to any one of claims 17-22 wherein the negative selection marker is selected from

a. a restriction enzyme recognition site not contained elsewhere in the first component or within the TCR joining gene segment,
b. a bacterial suicide gene, and
c. a reporter element,

and wherein the negative selection markers defined in b. of claim 18 and in c. of claim 19 are different from the negative selection marker defined in g. of claim 1, and the negative selection marker defined in g. of claim 1 is different from those in the V-C entry vector of the first component and the V-C entry vector encoding saida second TCR chain complimentary to said first TCR chain.

24. A library of V-C entry vectors as defined in any of claims 17-23, wherein the library comprises a first and second V-C entry vector library, each containing

a collection of one or more vectors representing all germline TCR variable and constant gene segments of an organism having such TCRs, or

a collection of one or more vectors representing a collection of variant germline TCR variable and constant gene segments of an organism having such TCRs, such that translated amino acid sequence of the encoded protein is unmodified in relation to the protein sequence encoded by the germline gene segment, or

a collection of one or more vectors representing a collection of variant germline TCR variable and constant gene segments of an organism having such TCRs, such that translated amino acid sequence of the encoded protein is modified in relation to the protein sequence encoded by the germline gene segment,

wherein the first and second V-C entry vector libraries encode reciprocal TCR chains.

25. A library of V-C entry vectors according to claim 24 for use to provide a pair of full length TCR open reading frames (ORFs), encoded in a antiparallel arrangement in a single product construct.

26. A method for in vitro reconstitution of a pair of full length TCR open reading frames (ORFs), encoded in an antiparallel arrangement in a single product construct, said method comprising

a. selecting a V-C entry vector for each TCR chain,
b. selecting a J donor vector for each TCR chain,
c. selecting an odeCDR3 for each TCR chain,
d. combining a, b and c for each TCR in an independent reaction to react with i) Type IIS restriction enzyme(s) to cleave the first to fourth Type IIS restriction enzyme recognition and cleavage sites present in the V-C entry vectors and in the J donor vectors and ii) DNA ligase enzyme and subjecting the combined mixes to thermocycling reactions to obtain a first and second reaction product,
e. combining both reaction products from step d. with a BiT donor vector as defined in claim 17 with i) Type IIS restriction enzyme(s) to cleave the fifth to tenth Type IIS restriction enzyme recognition and cleavage sites and ii) DNA ligase enzyme, and subjecting the combined reaction prod-

ucts to thermocycling reactions to obtain a third reaction product,

f. transforming the third reaction product from step e. into a host organism competent for DNA vector propagation, and

g. propagating said host organism to obtain full length reconstituted TCR open reading frame in the resulting V-C entry vector backbone.

27. A pair of V-C entry vectors represented by SEQ ID NO: 0756 and 0764.

28. A pair of V-C entry vectors according to claim 27, for use in recombinase mediated cassette exchange with genetic targets with matched heterospecific recombinase sequences.

29. A bidirectional terminator element, represented by the sequence SEQ0777.