Claims

~~1. A combined system comprising two separate components, wherein the first component is a tag-exchange donor vector (TEDV) encoding a first cell surface tag (CST) exon flanked by a 3' intron fragment, and a gene of interest (GOI) in the antisense orientation, the TEDV containing no promoter sequences, and the second component is an engineered cell containing within its genome a tag-exchange receiver site (TERS), encoding a second CST exon, which is different to the first CST, adjoined by a full intron sequence to an exon encoding a transmembrane domain, and also encoding a reporter gene in the antisense orientation, wherein paired recombinase mediated cassette exchange (RMCE) elements are included in the TEDV and TERS such that execution of RMCE between the TEDV and TERS results in exchange of the reporter element for the GOI encoded by the TEDV, and exchange of the first CST exon for the second CST exon, such that the derivative engineered cell now expresses the first CST and GOI, in place of the second CST and the reporter gene.~~

~~2. The combined system according to claim 1 wherein the first component is a TEDV comprising~~

~~a. a first RMCE element~~
~~b. a 3' intron fragment~~
~~c. a CST exon~~
~~d. a first transcriptional terminator~~
~~e. a second transcriptional terminator~~
~~f. a GOI~~
~~g. a Kozak sequence~~
~~h. a second RMCE element~~

~~wherein the CST exon and first transcriptional terminator are encoded in the antisense orientation from the GOI and associated transcriptional terminator and Kozak sequences.~~

~~3. The combined system according to claim 1, wherein the second component is a TERS comprising~~

~~a. a transcriptional promoter element~~
~~b. a Kozak sequence~~
~~c. a transmembrane domain exon~~
~~d. an intron~~

e. a first RMCE element encoded within the intron sequence d.

f. a CST exon

g. a first transcriptional terminator

h. a second transcriptional terminator i. a reporter gene

j. a Kozak sequence

k. a second RMCE element

l. a second transcriptional promoter element

wherein the transmembrane domain exon and CST exon are encoded in the antisense orientation from the reporter gene, such that the first transcriptional promoter element drives transcription of the combined transmembrane domain and CST, and the second transcriptional promoter element drives transcription of the reporter gene.

4. The combined system according to claim 2, wherein the second component is a TERS comprising

a. a transcriptional promoter element

b. a Kozak sequence

c. a transmembrane domain exon

d. an intron

e. a first RMCE element encoded within the intron sequence d.

f. a CST exon

g. a first transcriptional terminator

h. a second transcriptional terminator

i. a reporter gene

j. a Kozak sequence

k. a second RMCE element

l. a second transcriptional promoter element

wherein the transmembrane domain exon and CST exon are encoded in the antisense orientation from the reporter gene, such that the first transcriptional promoter element drives transcription of the combined transmembrane domain and CST, and the second transcriptional promoter element drives transcription of the reporter gene.

5. The combined system according to any of claims 1 to 4, wherein the first RMCE element of the TEDV is paired with the first RMCE element of the TERS, and the second RMCE element of the TEDV is paired with the second RMCE element of the TERS.

~~6. The combined system according to any of claims 1 to 5, wherein each CST exon comprises a sequence encoding one or more molecular affinity tags and that the CST encoded by the TEDV and TERS are different.~~

~~7. The combined system according to any of claims 1 to 5, wherein the engineered cell contains a single TERS in its genome.~~

~~8. A method for generating derivative engineered cells expressing a TEDV-encoded GOI from the TERS locus, said method comprising,~~

~~a. generating a TEDV encoding a GOI and containing no promoter sequences~~

~~b. delivering said TEDV to an engineered cell line containing a paired TERS, along with the recombinase enzyme matching the RMCE elements encoded therein~~

~~c. contacting cells with two or more affinity reagents specific for both the TEDV-encoded CST and TERS-encoded CST~~

~~d. selecting derivative engineered cells on the basis of diminished expression of the reporter gene and TERS-encoded CST, and increased expression of the TEDV-encoded CST, as a proxy for selection of cells with integrated GOI.~~

~~9. The method according to claim 8, wherein the affinity reagents used in step c. are fluorescently labelled as to detect diminished expression of TERS-encoded CST and increased expression of TEDV-encoded CST, as to enable cell partitioning and selection based on said expression by way of florescence activated cell sorting.~~

~~10. The method according to claim 8, wherein the affinity reagents used in step c. are immobilised to a substrate such that cells expressing TERS-encoded CST may be depleted or that cells expressing TEDV-encoded CST may be enriched, in the target cell population using substrate affinity methods, such as magnetic activated cell sorting.~~

~~11. A method for generating multiple derivative engineered cells expressing a range of TEDV-encoded GOI, from a pool of TEDV, said method comprising,~~

a. generating a library of two or more TEDV, each encoding a unique GOI sequence, each with a unique TEDV-encoded CST, and each containing no promoter sequences

b. delivering said library of TEDV as a pool, to an engineered cell line containing a paired TERS, along with the recombinase enzyme matching the RMCE elements encoded therein

c. contacting cells with three or more affinity reagents specific for both the multiple TEDV-encoded CST and TERS-encoded CST

d. selecting derivative engineered cells on the basis of diminished expression of the reporter gene and TERS-encoded CST, and increased expression of each of the unique TEDV-encoded CST

12. A method for cell lineage tracing of derivative engineered cells expressing a range of TEDV-encoded GOI, within a pool of cells as generated by the steps:

- generating a library of two or more TEDV, each encoding a unique GOI sequence, each with a unique TEDV-encoded CST, and each containing no promoter sequences

- delivering said library of TEDV as a pool, to an engineered cell line containing a paired TERS, along with the recombinase enzyme matching the RMCE elements encoded therein,

said method comprising,

a. contacting cells with two or more affinity reagents specific for the multiple TEDV-encoded CSTs

b. analysing the content of derivative engineered cells on the basis of expression of each of the unique TEDV-encoded CSTs.

13. A tag-exchange donor vector (TEDV) encoding a cell surface tag (CST) exon flanked by a 3' intron fragment, a gene of interest (GOI) in the antisense orientation, and containing no promoter sequences.

14. The tag-exchange donor vector (TEDV) according to claim 13, comprising

a. a first RMCE element

b. a 3' intron fragment

c. a CST exon

d. a first transcriptional terminator

e. a second transcriptional terminator

f. a GOI

g. a Kozak sequence

h. a second RMCE element

wherein the CST exon and first transcriptional terminator are encoded in the antisense orientation from the GOI and associated transcriptional terminator and Kozak sequences.

15. An engineered cell containing within its genome a tag-exchange receiver site (TERS), encoding a cell surface tag (CST) exon adjoined by a full intron sequence to an exon encoding a transmembrane domain, and also encoding a reporter gene in the antisense orientation, wherein recombinase mediated cassette exchange (RMCE) elements are included in the TERS such that execution of RMCE between TERS and a tag-exchange donor vector (TEDV) containing no promoter sequences results in exchange of the reporter element for a gene of interest (GOI) encoded by the TEDV.

16. The engineered cell according to claim 15, wherein said TERS comprises:

a. a transcriptional promoter element

b. a Kozak sequence

c. a transmembrane domain exon

d. an intron

e. a first RMCE element

f. a CST exon

g. a first transcriptional terminator

h. a second transcriptional terminator

i. a reporter gene

j. a Kozak sequence

k. a second RMCE element

l. a second transcriptional promoter element,

wherein the transmembrane domain exon and CST exon are encoded in the antisense orientation from the reporter gene, such that the first transcriptional promoter element drives transcription of the combined transmembrane domain and CST, and the second transcriptional promoter element drives transcription of the reporter gene.

1. A combined system comprising two separate components, wherein the first component is a tag-exchange donor vector (TEDV) encoding a first cell surface tag (CST) exon flanked by a 3' intron fragment, and a gene of interest (GOI) in the antisense orientation, the TEDV containing no promoter sequences, and the second component is an engineered cell containing within its genome a tag-exchange receiver site (TERS), encoding a second CST exon, which is different to the first CST, adjoined by a full intron sequence to an exon encoding a transmembrane domain, and also encoding a reporter gene in the antisense orientation, wherein paired recombinase mediated cassette exchange (RMCE) elements are included in the TEDV and TERS such that execution of RMCE between the TEDV and TERS results in exchange of the reporter element for the GOI encoded by the TEDV, and exchange of the first CST exon for the second CST exon, such that the derivative engineered cell now expresses the first CST and GOI, in place of the second CST and the reporter gene,

wherein the first component is a TEDV comprising from 5' to 3'

a. a first 5' RMCE element encoded in the non-coding and non-functional 3' intron fragment

b. a 3' intron fragment

c. an exon comprising TEDV-encoded CST in the 5' to 3' direction

d. a first transcriptional terminator sequence for encoded CST in the 5' to 3' direction

e. a second transcriptional terminator sequence for the 3' to 5' encoded GOI

f. a sequence encoding the GOI in the 3' to 5' direction

g. a Kozak sequence

h. a second 3' RMCE element

wherein the CST exon and first transcriptional terminator are encoded in the antisense orientation from the GOI and from the associated transcriptional terminator and Kozak sequences, and

wherein the second component is a TERS comprising from 5' to 3'

a. a transcriptional promoter element

b. a Kozak sequence

c. an exon encoding a protein transmembrane domain

d. a 5' intron

e. a first 5' RMCE element encoded within the intron sequence d and paired with 5' RMCE element of TEDV.

f. an exon comprising TERS-encoded CST in the 5' to 3' direction

g. a first transcriptional terminator sequence for encoded CST in the 5' to 3' direction

h. a second transcriptional terminator sequence for the 3' to 5' encoded selection gene

i. a sequence encoding a reporter gene in the 3' to 5' direction

j. a Kozak sequence

k. a second 3' RMCE element paired with the 3' RMCE element of TEDV

l. a second transcriptional promoter element

wherein the transmembrane domain exon and CST exon are encoded in the antisense orientation from the reporter gene, such that the first transcriptional promoter element drives transcription of the combined transmembrane domain and CST, and the second transcriptional promoter element drives transcription of the reporter gene.

2. The combined system according to claim 1, wherein the first RMCE element of the TEDV is paired with the first RMCE element of the TERS, and the second RMCE element of the TEDV is paired with the second RMCE element of the TERS.

3. The combined system according to claim 1 or 2, wherein each CST exon comprises a sequence encoding one or more molecular affinity tags and that the CST encoded by the TEDV and TERS are different.

4. The combined system according to any of claims 1 to 3, wherein the engineered cell contains a single TERS in its genome.

5. Use of the combined system as defined in any one of claims 1 to 4 for generating derivative engineered cells, said derivative engineered cells expressing a TEDV-encoded GOI from the TERS locus, said method comprising,

a. generating a TEDV as defined in any of claims 1 to 4 and encoding a GOI and containing no promoter sequences

b. delivering said TEDV to an engineered cell line containing a paired TERS as defined in any of claims 1 to 4, along with a recombinase enzyme matching the RMCE elements encoded therein

c. contacting cells with two or more affinity reagents specific for both the TEDV-encoded CST and the TERS-encoded CST

d. selecting derivative engineered cells on the basis of diminished expression of the reporter gene and TERS-encoded CST, and increased expression of the TEDV-encoded CST, as a proxy for selection of cells with integrated GOI.

6. Use according to claim 5, wherein the affinity reagents used in step c. are fluorescently labelled as to detect diminished expression of TERS-encoded CST and increased expression of TEDV-encoded CST, as to enable cell partitioning and selection based on said expression by way of florescence activated cell sorting.

7. Use according to claim 6, wherein the affinity reagents used in step c. are immobilised to a substrate such that cells expressing TERS-encoded CST may be depleted or that cells expressing TEDV-encoded CST may be enriched, in the target cell population using substrate affinity methods, such as magnetic activated cell sorting.

8. A method for generating multiple derivative engineered cells from the combined system as defined in any one of claims 1 to 4, said derivative engineered cells expressing a range of TEDV-encoded GOI, from a pool of TEDV, said method comprising,

a. generating a library of two or more TEDV as defined in any one of claims 1 to 4, each encoding a unique GOI sequence, each with a unique TEDV-encoded CST, and each containing no promoter sequences

b. delivering said library of TEDV as a pool, to an engineered cell line containing a paired TERS as defined in any one of claims 1 to 4, along with the recombinase enzyme matching the RMCE elements encoded therein

c. contacting cells with three or more affinity reagents specific for both the multiple TEDV-encoded CST and TERS-encoded CST

d. selecting derivative engineered cells on the basis of diminished expression of the reporter gene and TERS-encoded CST, and increased expression of each of the unique TEDV-encoded CST

9. A method for cell lineage tracing of derivative engineered cells obtained from the combined system as defined in any one of claims 1 to 4, said derivative engineered cells expressing a range of TEDV-encoded GOI, within a pool of cells as generated by the steps:

– generating a library of two or more TEDV as defined in any one of claims 14, each encoding a unique GOI sequence, each with a unique TEDV-encoded CST, and each containing no promoter sequences

– delivering said library of TEDV as a pool, to an engineered cell line containing a paired TERS as defined in any one of claims 1 to 4, along with the recombinase enzyme matching the RMCE elements encoded therein, said method comprising,

a. contacting cells with two or more affinity reagents specific for the multiple TEDV-encoded CSTs

b. analysing the content of derivative engineered cells on the basis of expression of each of the unique TEDV-encoded CSTs.

10. A tag-exchange donor vector (TEDV) as defined in any one of claims 1 to 4 encoding a cell surface tag (CST) exon flanked by a 3' intron fragment, a gene of interest (GOI) in the antisense orientation, and containing no promoter sequences,

wherein said TEDV comprises from 5' to 3'

a. a first 5' RMCE element encoded in the non-coding and non-functional 3' intron fragment

b. a 3' intron fragment

c. an exon comprising TEDV-encoded CST in the 5' to 3' direction

d. a first transcriptional terminator sequence for encoded CST in the 5' to 3' direction

e. a second transcriptional terminator sequence for the 3' to 5' encoded GOI

f. a sequence encoding the GOI in the 3' to 5' direction

g. a Kozak sequence

h. a second 3' RMCE element

wherein the CST exon and first transcriptional terminator are encoded in the antisense orientation from the GOI and from the associated transcriptional terminator and Kozak sequences.

11. An engineered cell containing within its genome a tag-exchange receiver site (TERS) as defined in any of claims 1 to 4, encoding a cell surface tag (CST) exon adjoined by a full intron sequence to an exon encoding a transmembrane domain, and also encoding a reporter gene in the antisense orientation, wherein recombinase mediated cassette exchange (RMCE) elements are included in the TERS such that execution of RMCE between TERS and a tag-exchange donor vector (TEDV) as defined in any one of claims 1-4 or 10 results in exchange of the reporter element for a gene of interest (GOI) encoded by the TEDV.

12. The engineered cell according to claim 11, wherein said TERS comprises from 5' to 3':

a. a transcriptional promoter element

b. a Kozak sequence

c. an exon encoding a protein transmembrane domain

d. a 5' intron

e. a first 5' RMCE element encoded within the intron sequence d

f. an exon comprising TERS-encoded CST in the 5' to 3' direction

g. a first transcriptional terminator sequence for encoded CST in the 5' to 3' direction

h. a second transcriptional terminator sequence for the 3' to 5' encoded selection gene

i. a sequence encoding a reporter gene in the 3' to 5' direction

j. a Kozak sequence

k. a second 3' RMCE element

l. a second transcriptional promoter element

wherein the transmembrane domain exon and CST exon are encoded in the antisense orientation from the reporter gene, such that the first transcriptional promoter element drives transcription of the combined transmembrane domain and CST, and the second transcriptional promoter element drives transcription of the reporter gene.