Figure 1

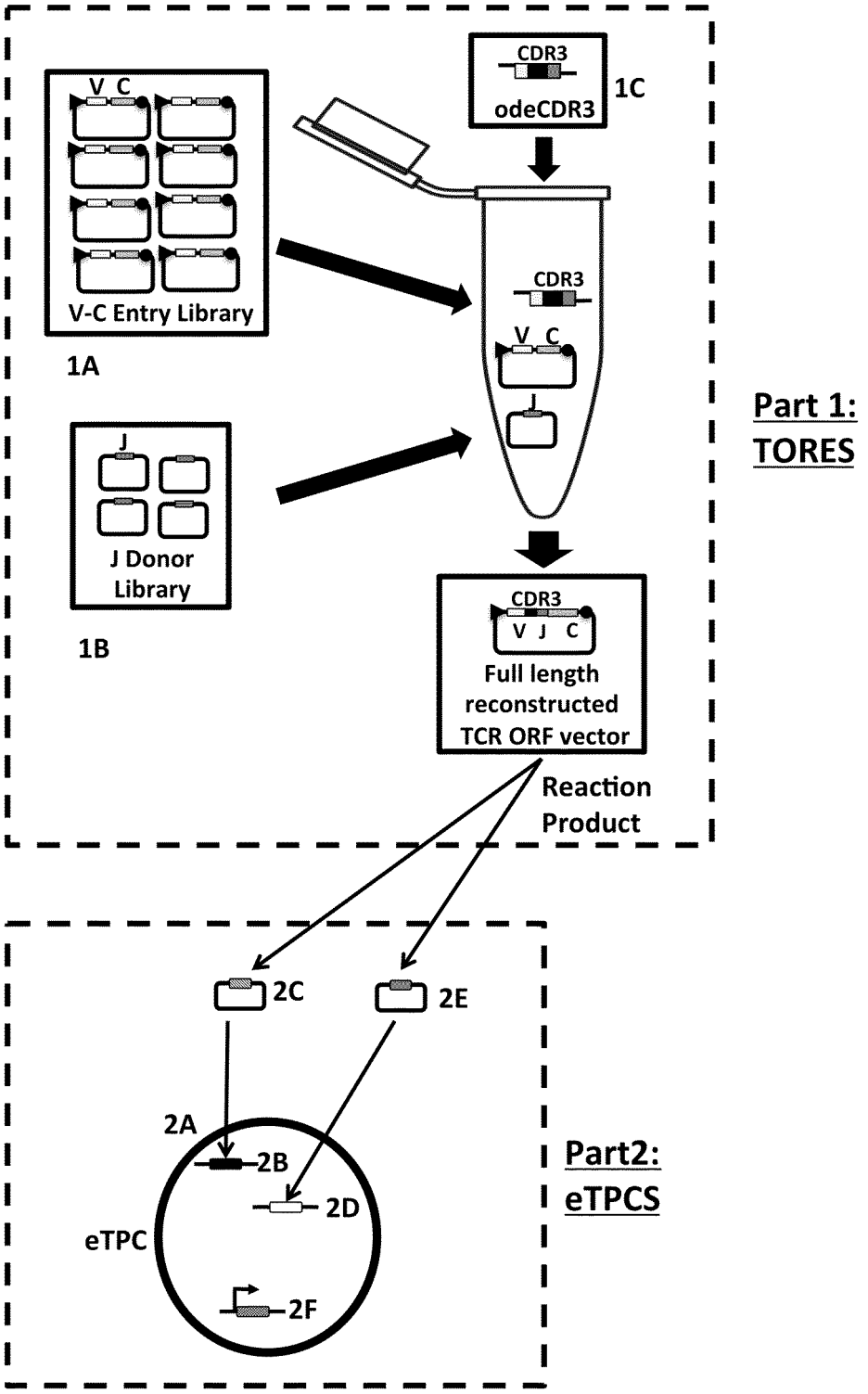

V C
V-C Entry Library
1A
J
J Donor Library
1B
CDR3
odeCDR3
1C
CDR3
V C
J
CDR3
V J C
Full length reconstructed TCR ORF vector
Reaction Product
Part 1: TORES
2C
2E
2A
2B
2D
eTPC
2F
Part2: eTPCS

Figure 2

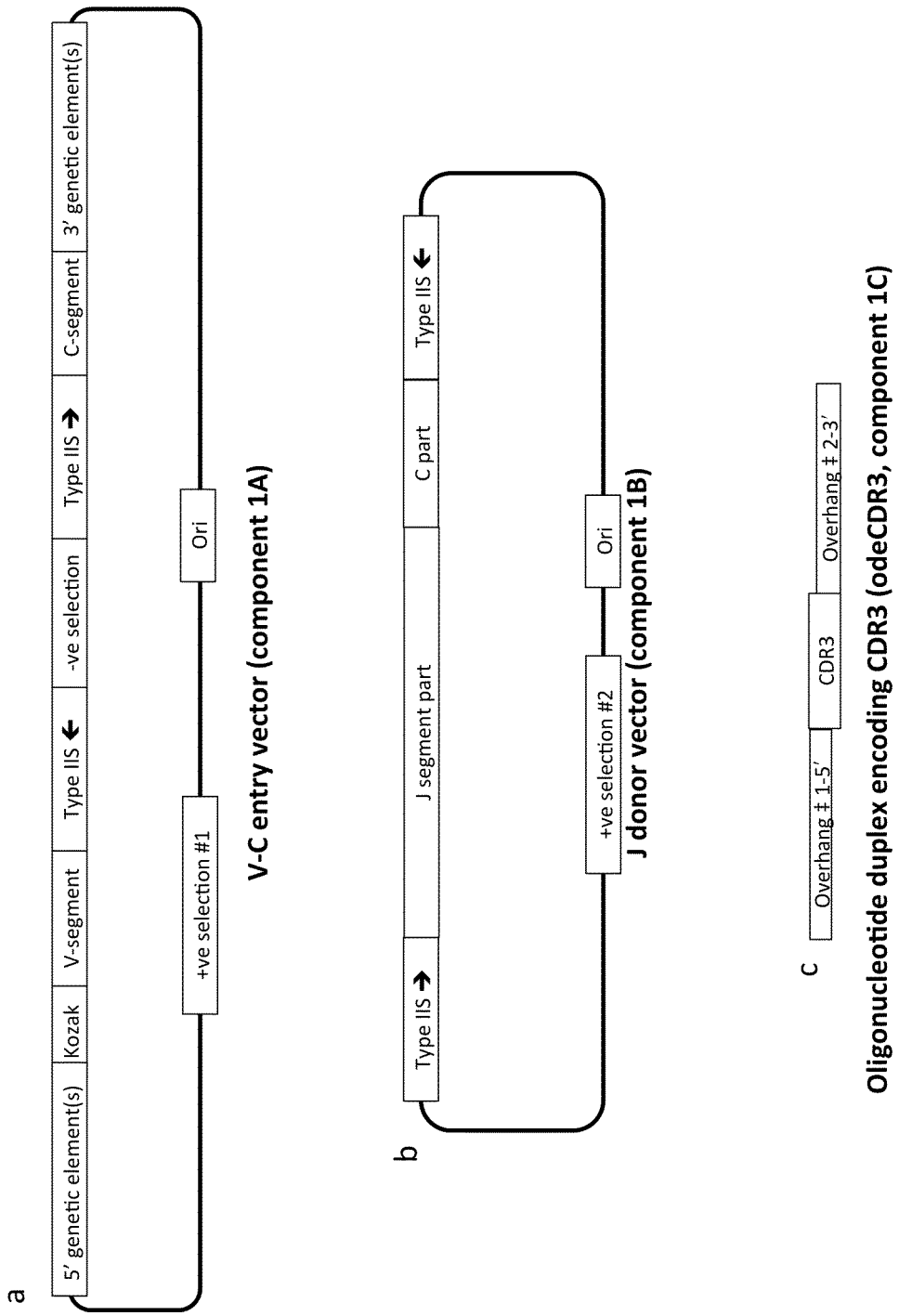

a
5' genetic element(s)
Kozak
V-segment
Type IIS
-ve selection
Type IIS
C-segment
3' genetic element(s)
+ve selection #1
Ori
V-C entry vector (component 1A)
b
Type IIS
J segment part
C part
Type IIS
+ve selection #2
Ori
J donor vector (component 1B)
c
Overhang ‡ 1-5'
CDR3
Overhang ‡ 2-3'
Oligonucleotide duplex encoding CDR3 (odeCDR3, component 1C)

Figure 2

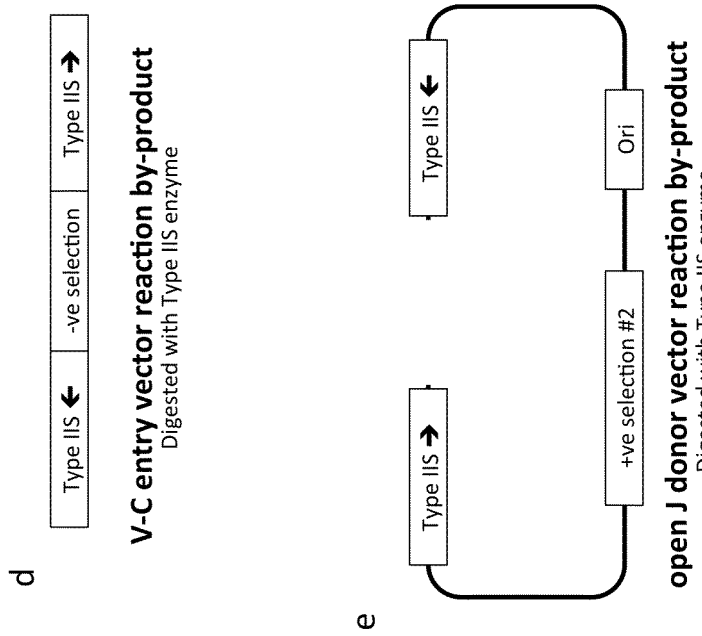

d
Type IIS
-ve selection
Type IIS
V-C entry vector reaction by-product
Digested with Type IIS enzyme
e
Type IIS
Type IIS
+ve selection #2
Ori
open J donor vector reaction by-product
Digested with Type IIS enzyme

Figure 2

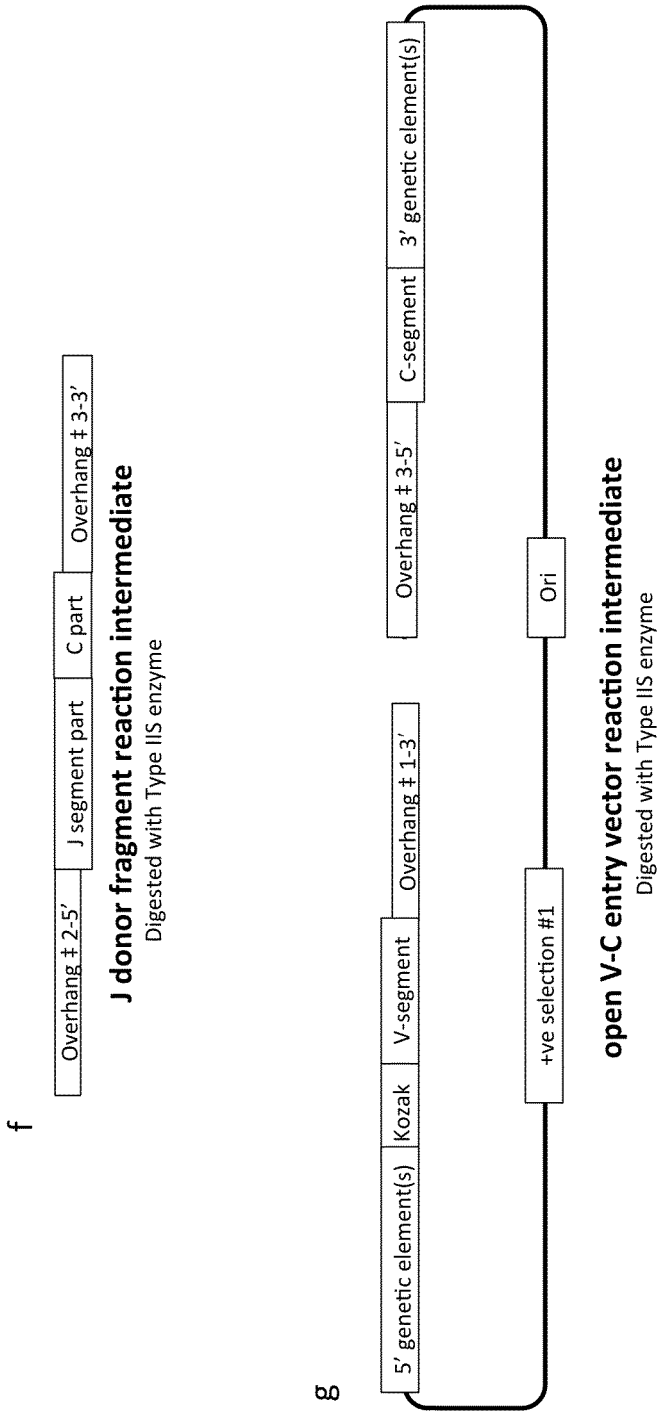

f
Overhang ‡ 2-5′
J segment part
C part
Overhang ‡ 3-3′
J donor fragment reaction intermediate
Digested with Type IIS enzyme
g
5′ genetic element(s)
Kozak
V-segment
Overhang ‡ 1-3′
+ve selection #1
Ori
Overhang ‡ 3-5′
C-segment
3′ genetic element(s)
open V-C entry vector reaction intermediate
Digested with Type IIS enzyme

Figure 2

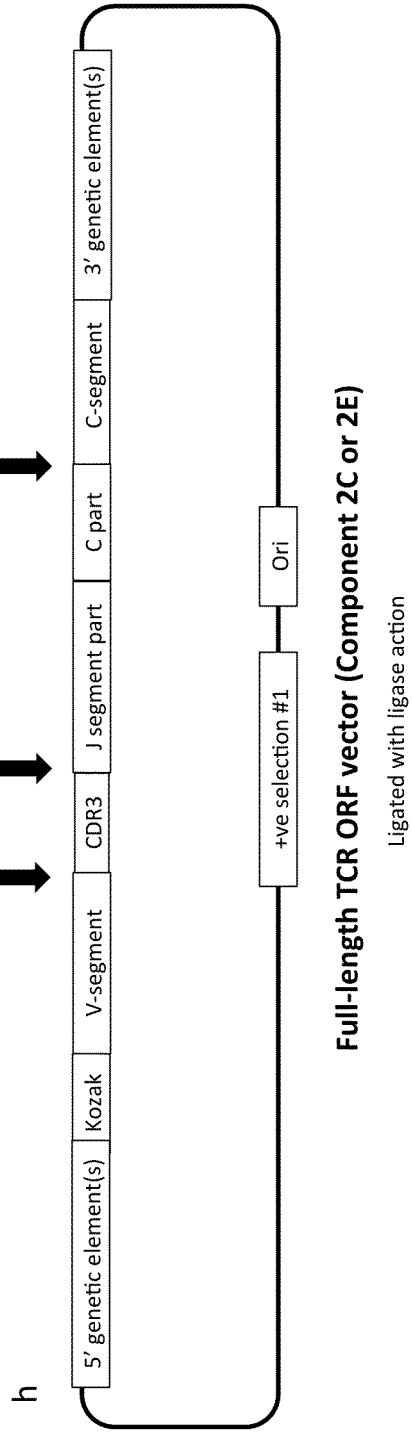

h
5' genetic element(s)
Kozak
V-segment
CDR3
J segment part
C part
C-segment
3' genetic element(s)
‡ 1
‡ 2
‡ 3
+ve selection #1
Ori
Full-length TCR ORF vector (Component 2C or 2E)
Ligated with ligase action

Figure 3

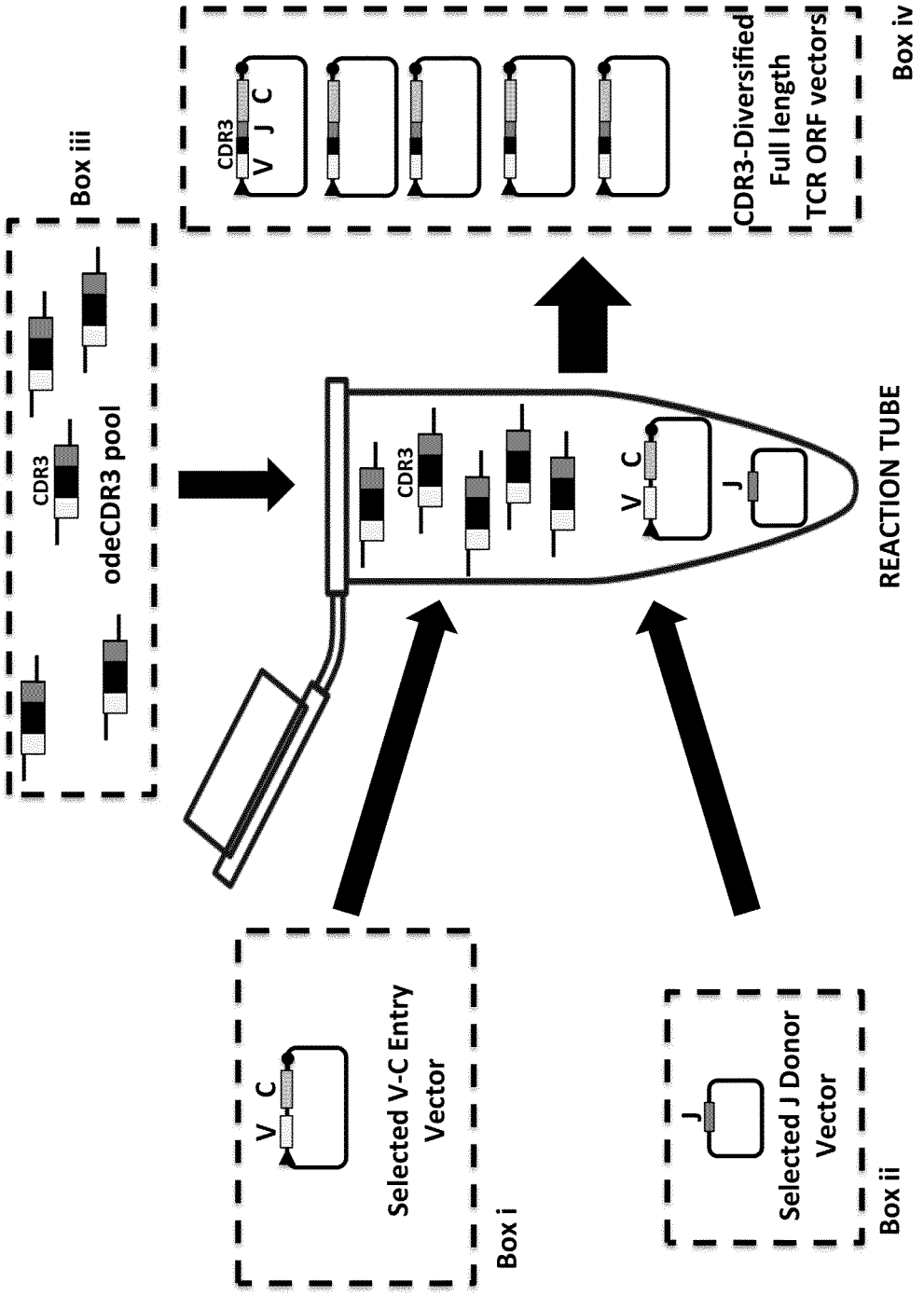

Box iii
odeCDR3 pool
CDR3
Box iv
CDR3-Diversified Full length TCR ORF vectors
CDR3
V J C
REACTION TUBE
CDR3
V C
J
Box i
Selected V-C Entry Vector
V C
Box ii
Selected J Donor Vector
J

Figure 4

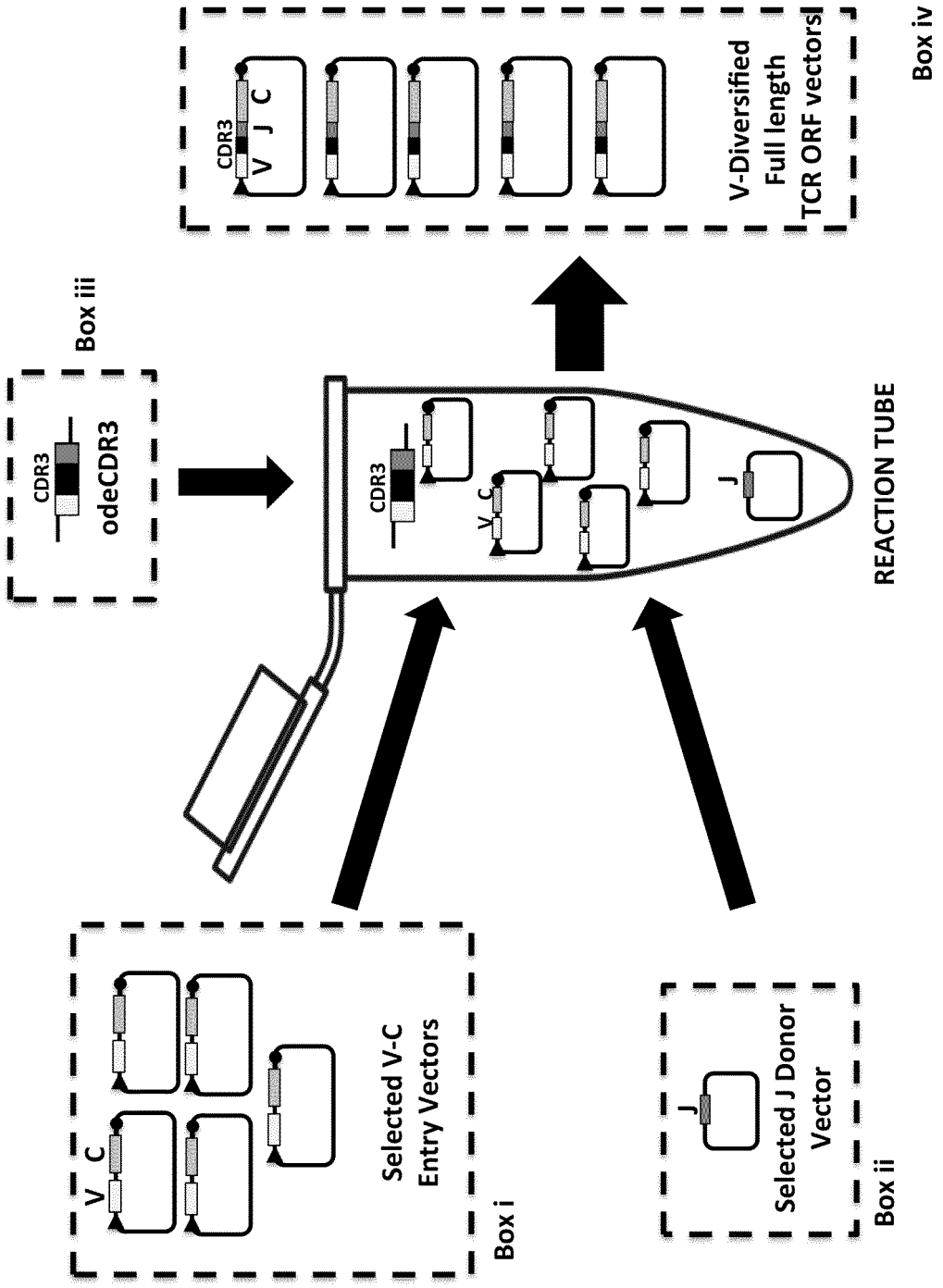

Box iv
V-Diversified Full length TCR ORF vectors
CDR3
V J C
Box iii
odeCDR3
CDR3
REACTION TUBE
CDR3
V C
J
Box i
Selected V-C Entry Vectors
V C
Box ii
Selected J Donor Vector
J

Figure 5

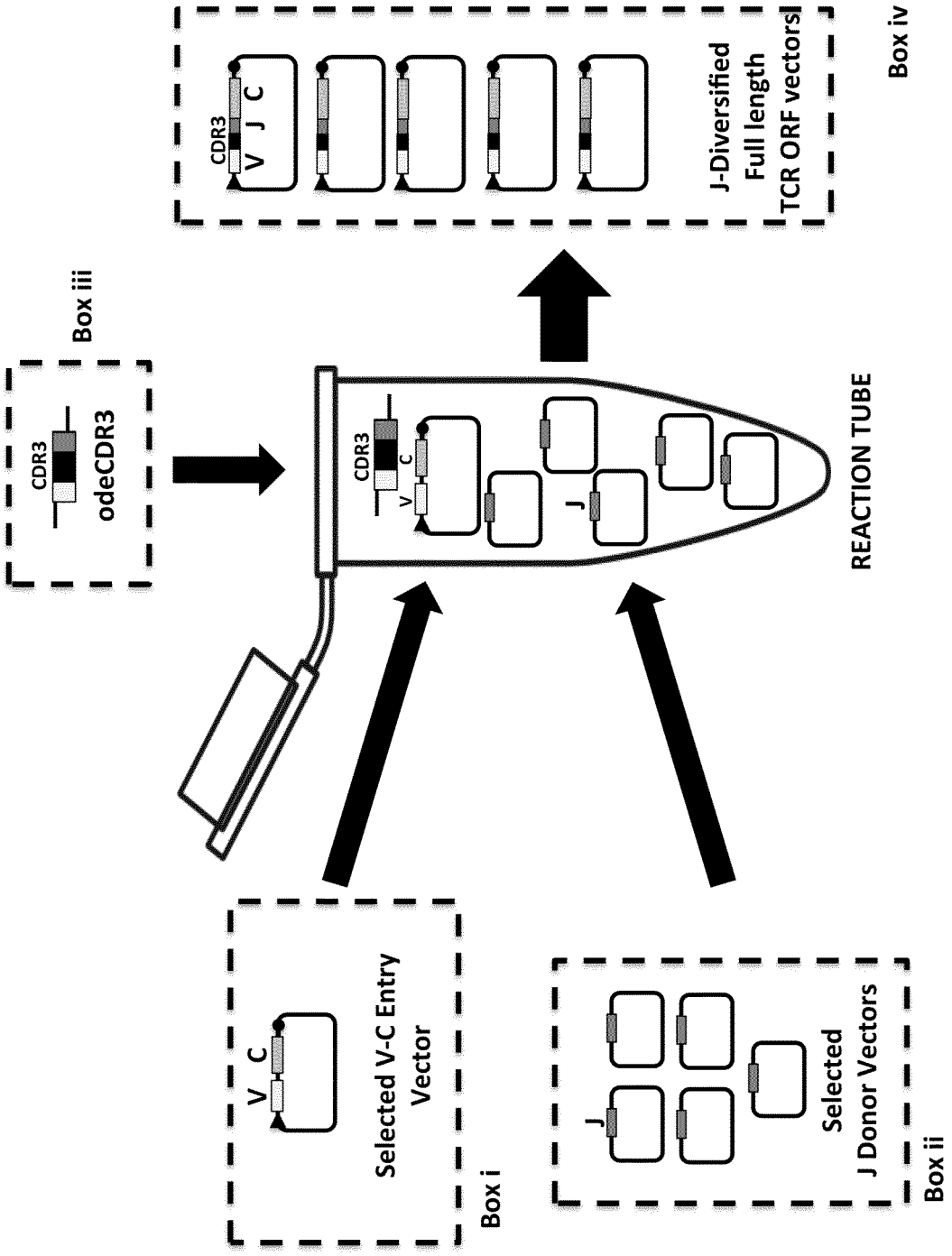

Box iii
CDR3
odeCDR3
CDR3
V J C
J-Diversified
Full length
TCR ORF vectors
Box iv
CDR3
V C
J
REACTION TUBE
V C
Selected V-C Entry
Vector
Box i
J
Selected
J Donor Vectors
Box ii

Figure 6

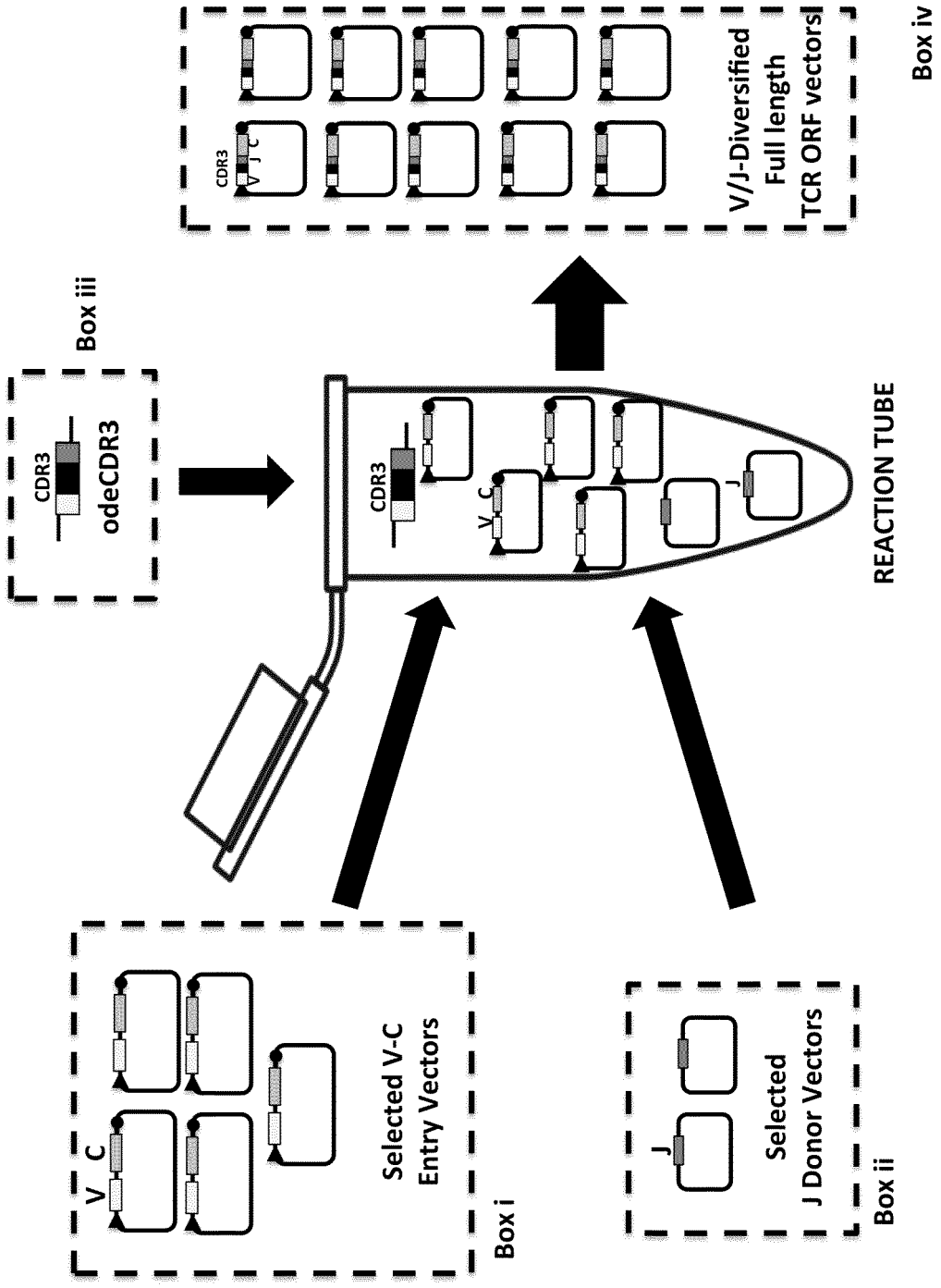

Box iv
V/J-Diversified
Full length
TCR ORF vectors
CDR3
V J C
Box iii
odeCDR3
CDR3
REACTION TUBE
V C
J
Box i
Selected V-C
Entry Vectors
V C
Box ii
Selected
J Donor Vectors
J

Figure 7

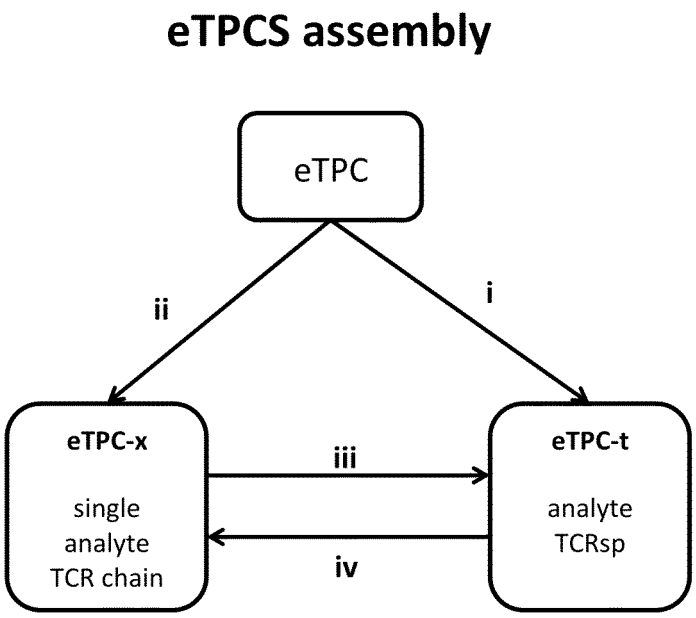

eTPCS assembly
eTPC
ii
i
eTPC-x
single
analyte
TCR chain
iii
iv
eTPC-t
analyte
TCRsp

Figure 8

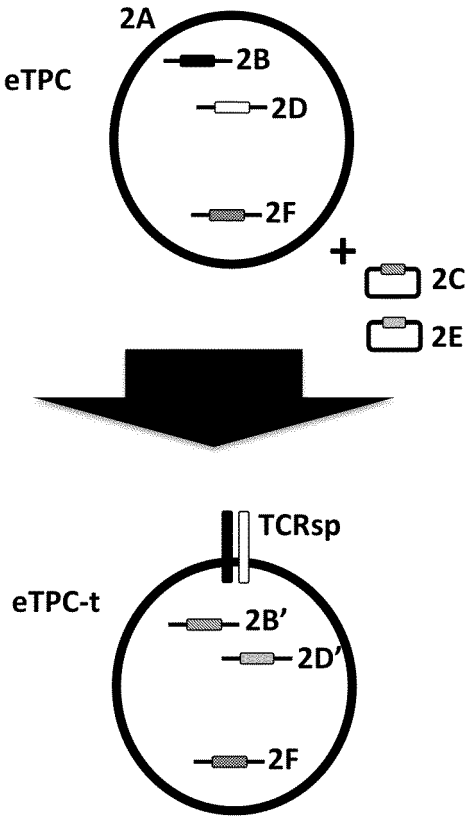


Figure 9

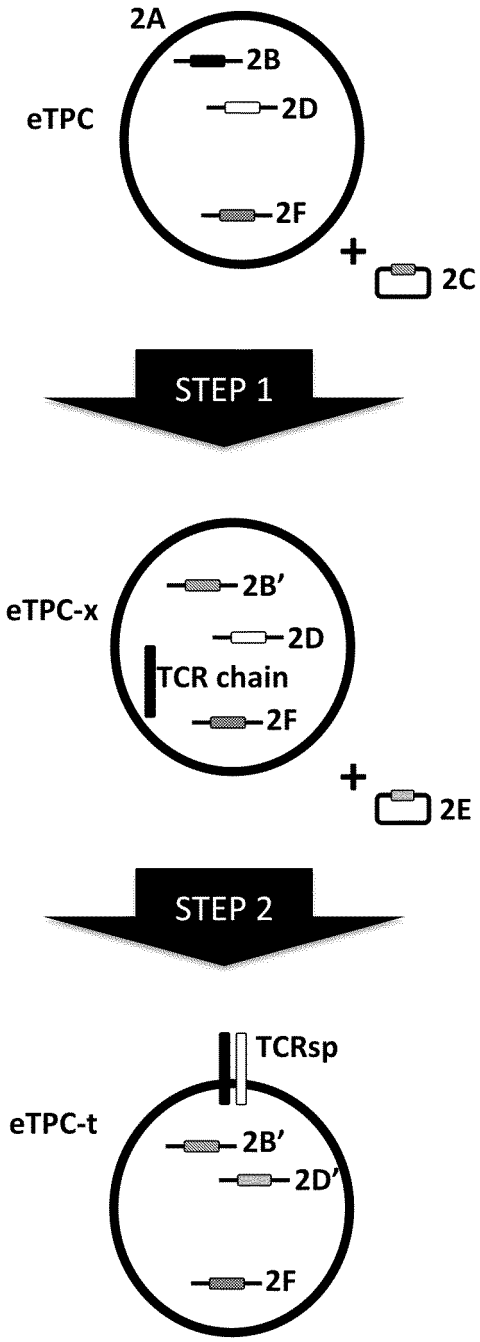

2A
2B
eTPC
2D
2F
+
2C
STEP 1
eTPC-x
2B'
2D
TCR chain
2F
+
2E
STEP 2
TCRsp
eTPC-t
2B'
2D'
2F

Figure 10

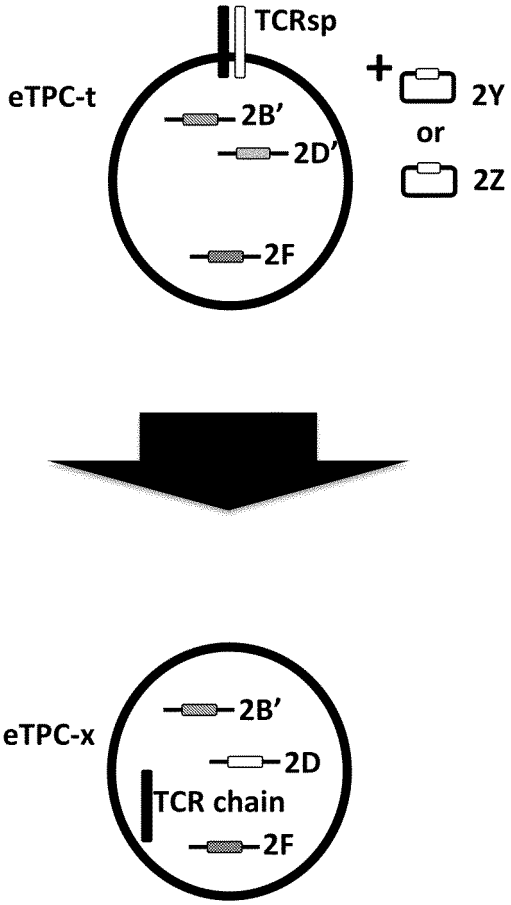


Figure 11

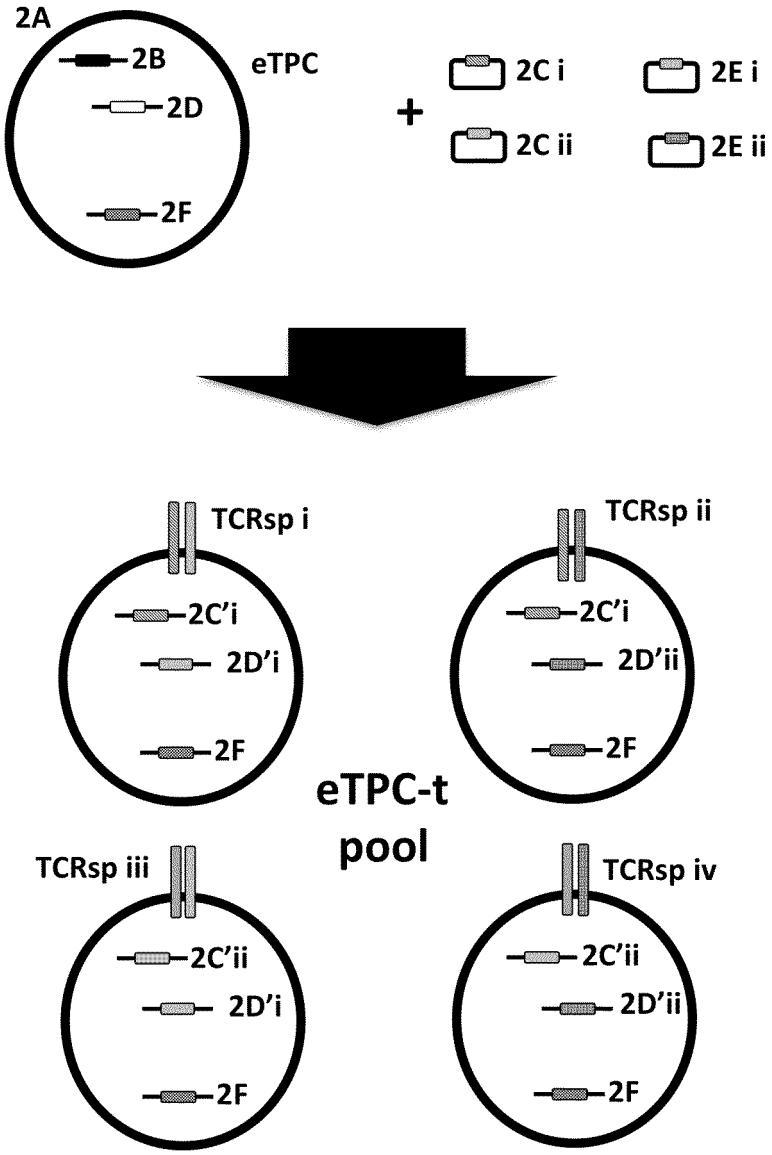

2A
2B
2D
2F
eTPC
+
2C i
2C ii
2E i
2E ii
TCRsp i
2C'i
2D'i
2F
TCRsp ii
2C'i
2D'ii
2F
eTPC-t pool
TCRsp iii
2C'ii
2D'i
2F
TCRsp iv
2C'ii
2D'ii
2F

Figure 12

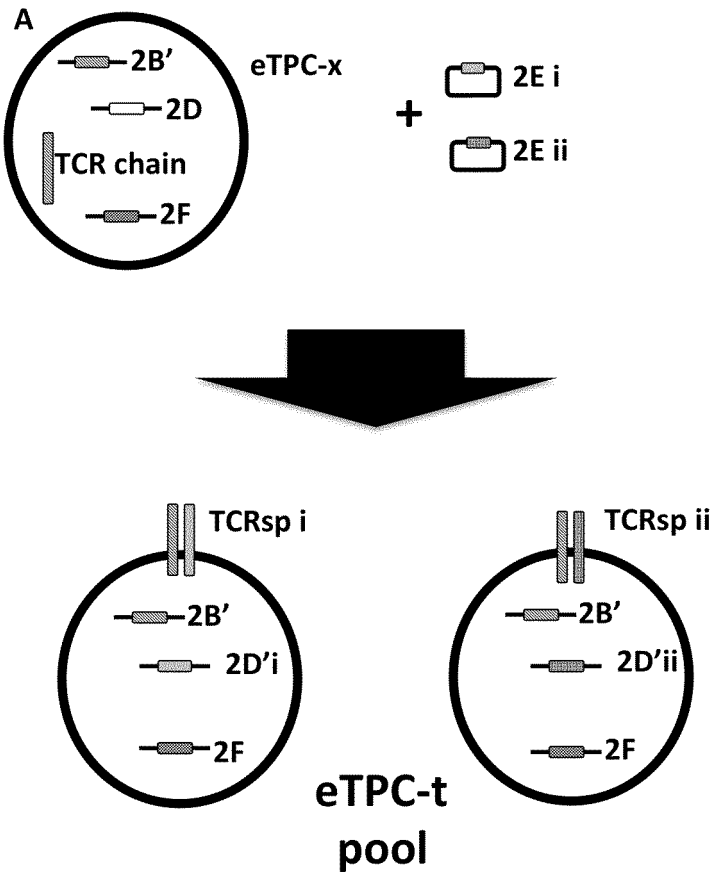

A
2B'
2D
TCR chain
2F
eTPC-x
+
2E i
2E ii
TCRsp i
2B'
2D'i
2F
TCRsp ii
2B'
2D'ii
2F
eTPC-t pool

Figure 13

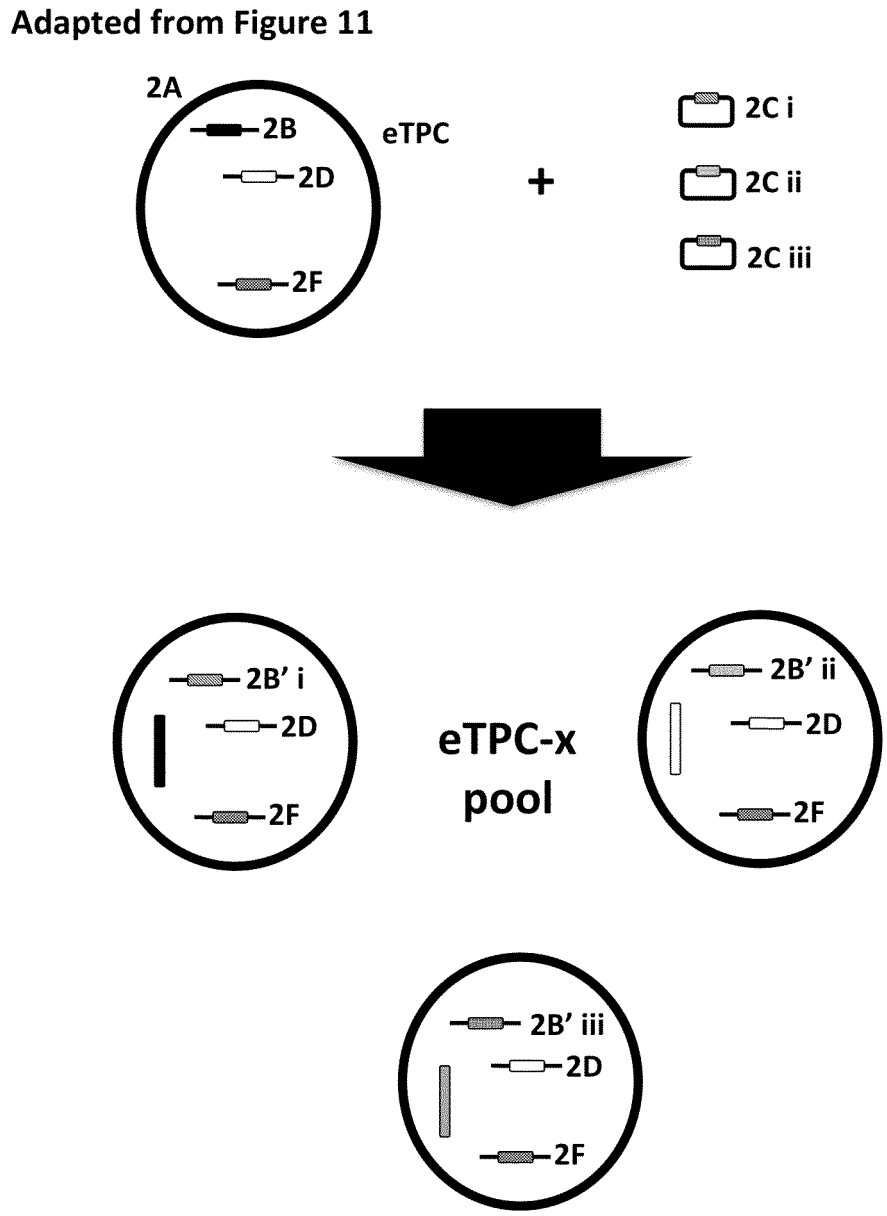

Adapted from Figure 11
2A
2B
2D
2F
eTPC
+
2C i
2C ii
2C iii
2B' i
2D
2F
eTPC-x pool
2B' ii
2D
2F
2B' iii
2D
2F

Figure 14

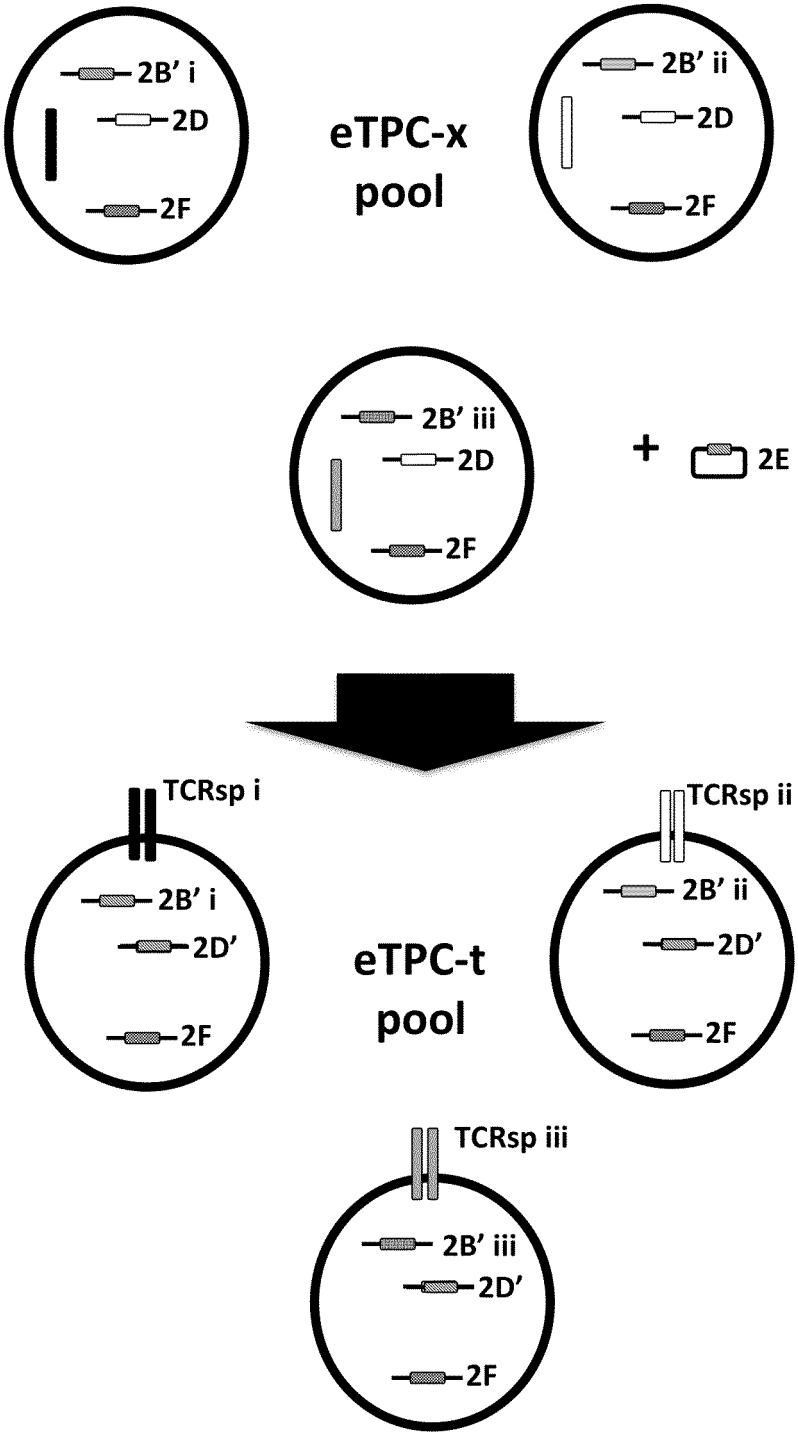

2B' i
2D
2F
eTPC-x pool
2B' ii
2D
2F
2B' iii
2D
2F
+
2E
TCRsp i
2B' i
2D'
2F
eTPC-t pool
TCRsp ii
2B' ii
2D'
2F
TCRsp iii
2B' iii
2D'
2F

Figure 15

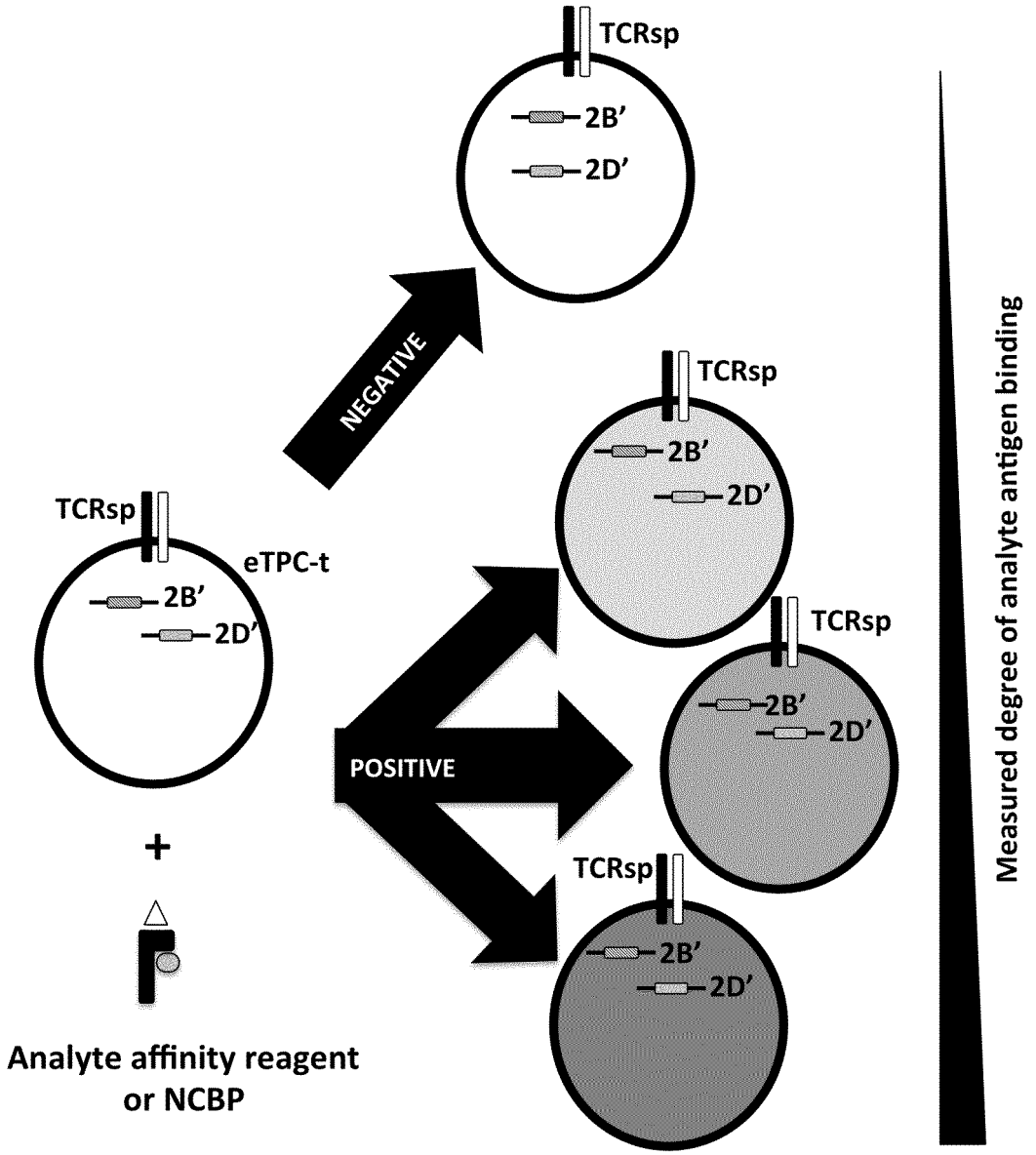

TCRsp
2B'
2D'
NEGATIVE
TCRsp
2B'
2D'
TCRsp
eTPC-t
2B'
2D'
TCRsp
2B'
2D'
POSITIVE
TCRsp
2B'
2D'
+
Analyte affinity reagent
or NCBP
Measured degree of analyte antigen binding

Figure 16

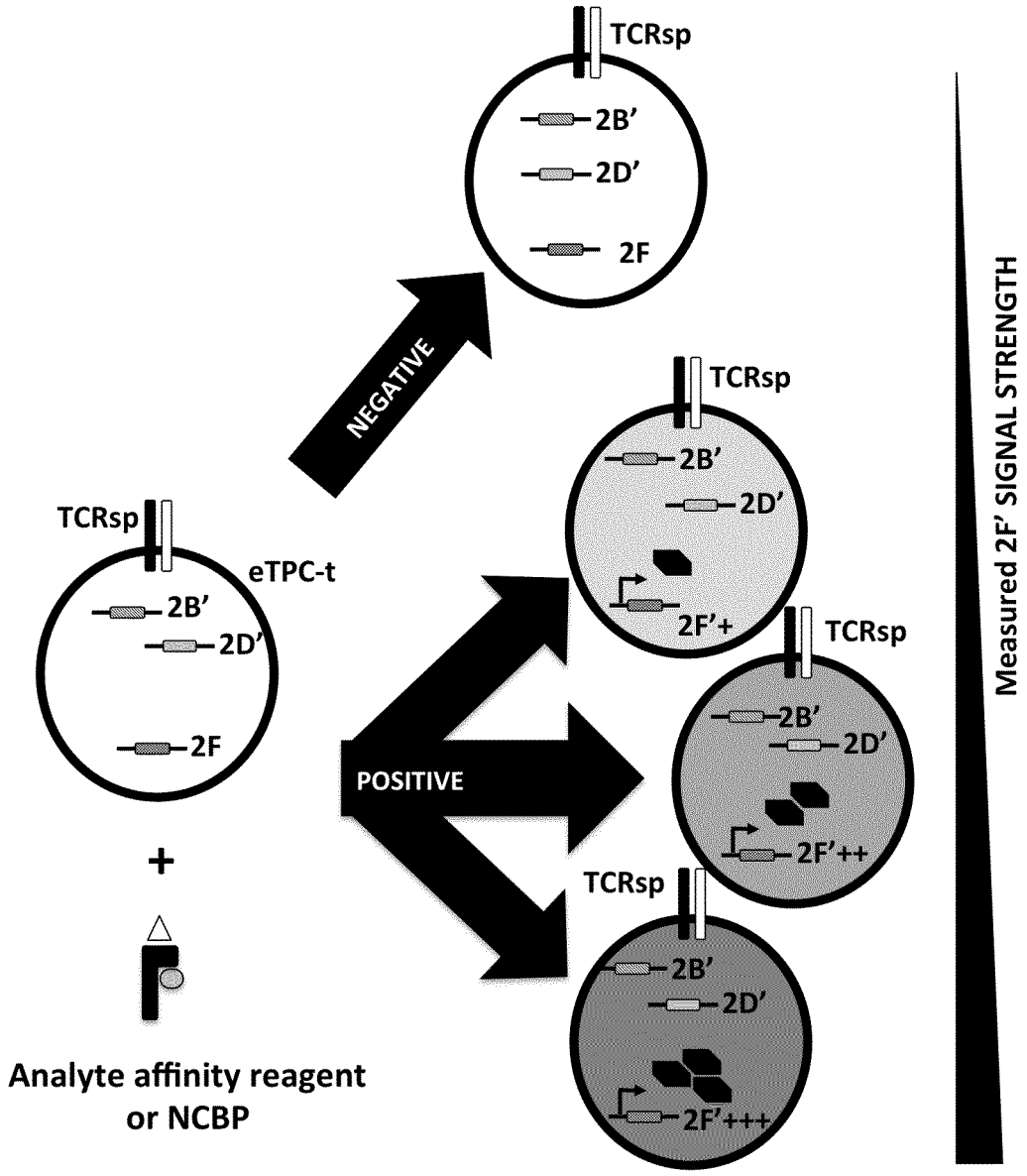

TCRsp
2B'
2D'
2F
NEGATIVE
TCRsp
2B'
2D'
2F'+
TCRsp
eTPC-t
2B'
2D'
2F
POSITIVE
TCRsp
2B'
2D'
2F'++
TCRsp
2B'
2D'
2F'+++
+
Analyte affinity reagent
or NCBP
Measured 2F' SIGNAL STRENGTH

Figure 17

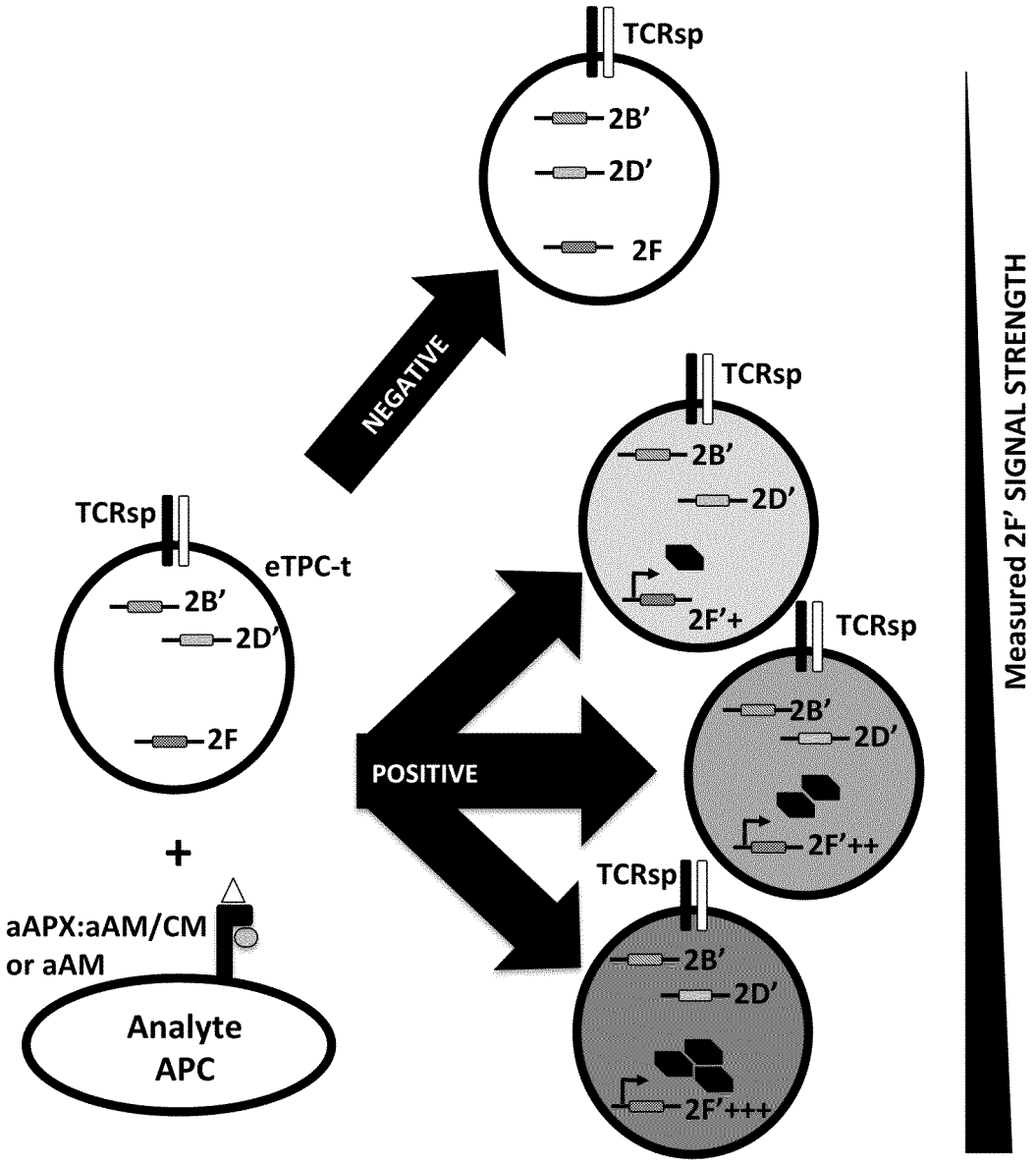

TCRsp
2B'
2D'
2F
NEGATIVE
TCRsp
eTPC-t
2B'
2D'
2F
+
aAPX:aAM/CM
or aAM
Analyte
APC
POSITIVE
TCRsp
2B'
2D'
2F'+
TCRsp
2B'
2D'
2F'++
TCRsp
2B'
2D'
2F'+++
Measured 2F' SIGNAL STRENGTH

Figure 18

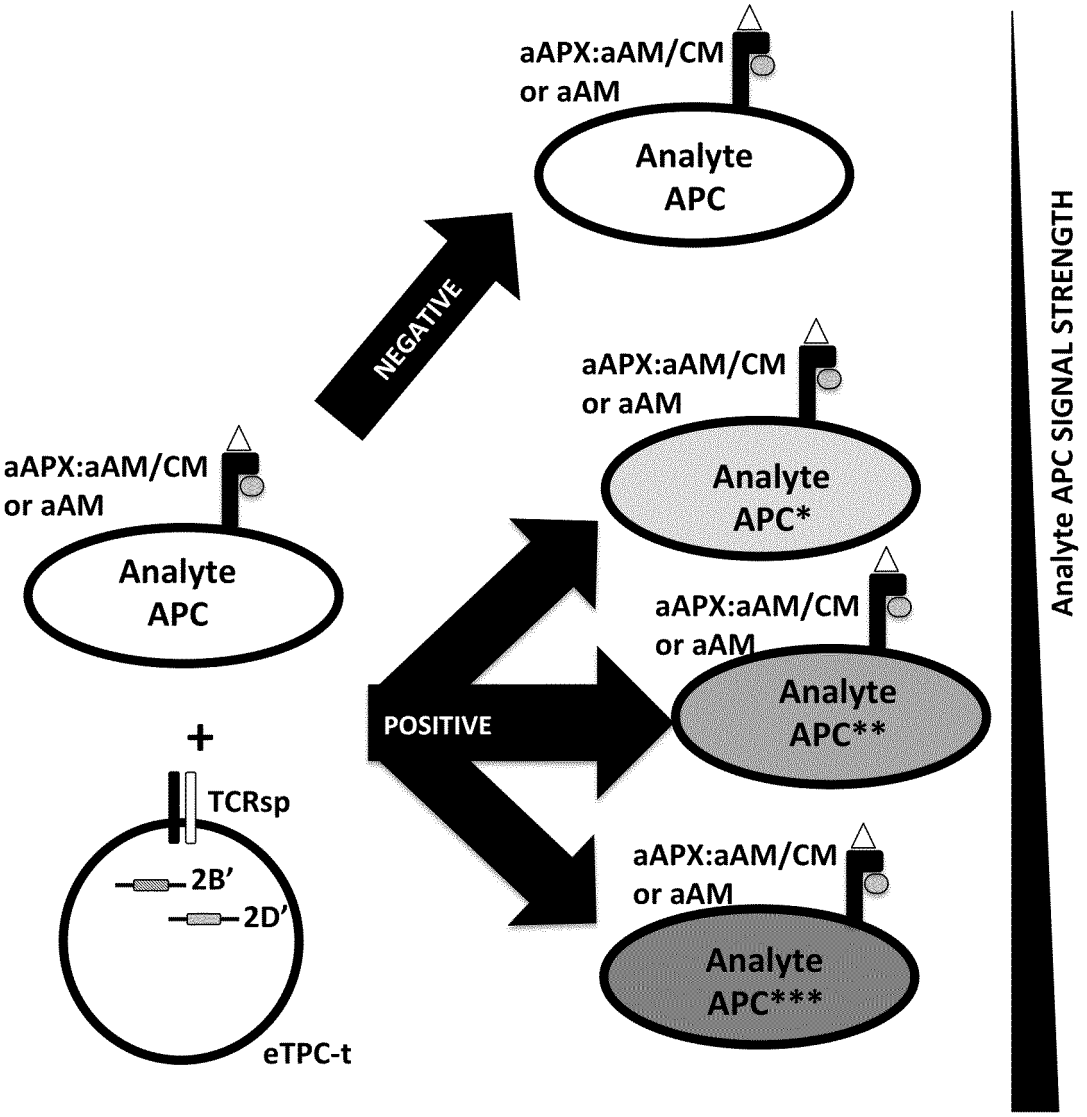

aAPX:aAM/CM
or aAM
Analyte
APC
NEGATIVE
aAPX:aAM/CM
or aAM
Analyte
APC*
aAPX:aAM/CM
or aAM
Analyte
APC
POSITIVE
aAPX:aAM/CM
or aAM
Analyte
APC**
+
TCRsp
2B'
2D'
aAPX:aAM/CM
or aAM
Analyte
APC***
eTPC-t
Analyte APC SIGNAL STRENGTH

Figure 19

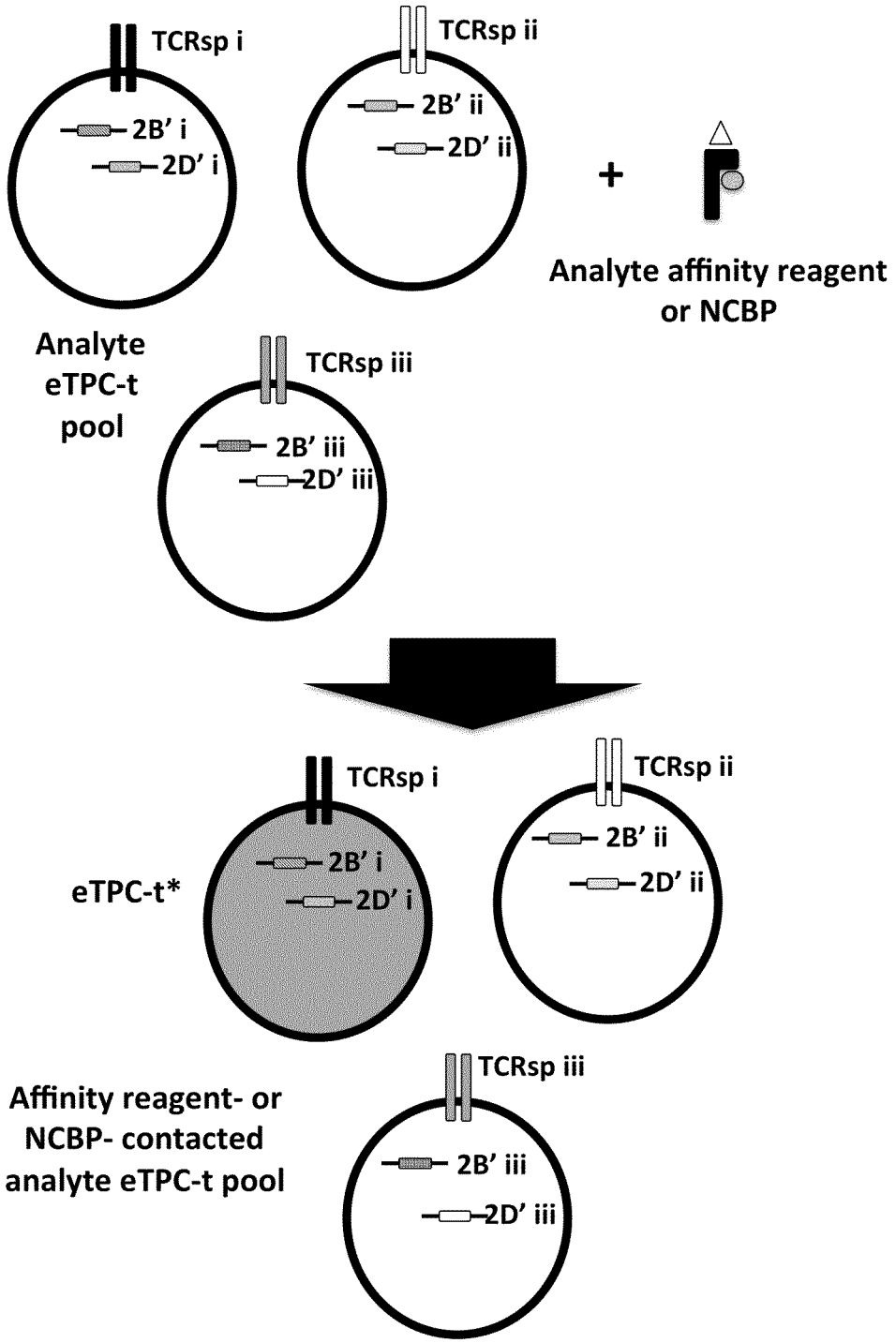

TCRsp i
2B' i
2D' i
TCRsp ii
2B' ii
2D' ii
+
Analyte affinity reagent or NCBP
Analyte eTPC-t pool
TCRsp iii
2B' iii
2D' iii
eTPC-t*
TCRsp i
2B' i
2D' i
TCRsp ii
2B' ii
2D' ii
Affinity reagent- or NCBP- contacted analyte eTPC-t pool
TCRsp iii
2B' iii
2D' iii

Figure 20

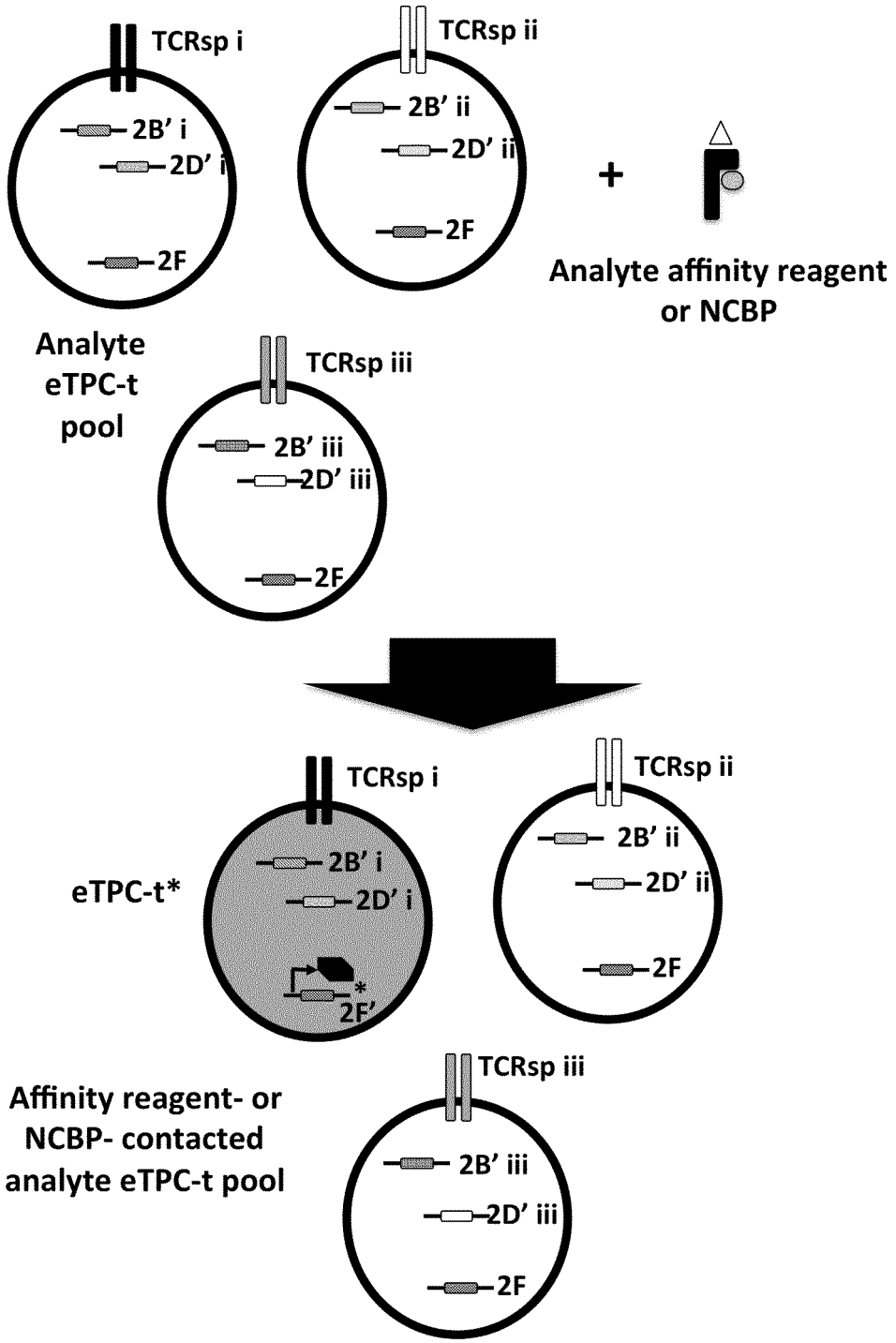

TCRsp i
2B' i
2D' i
2F
TCRsp ii
2B' ii
2D' ii
2F
+
Analyte affinity reagent or NCBP
Analyte eTPC-t pool
TCRsp iii
2B' iii
2D' iii
2F
eTPC-t*
TCRsp i
2B' i
2D' i
*
2F'
TCRsp ii
2B' ii
2D' ii
2F
Affinity reagent- or NCBP- contacted analyte eTPC-t pool
TCRsp iii
2B' iii
2D' iii
2F

Figure 21

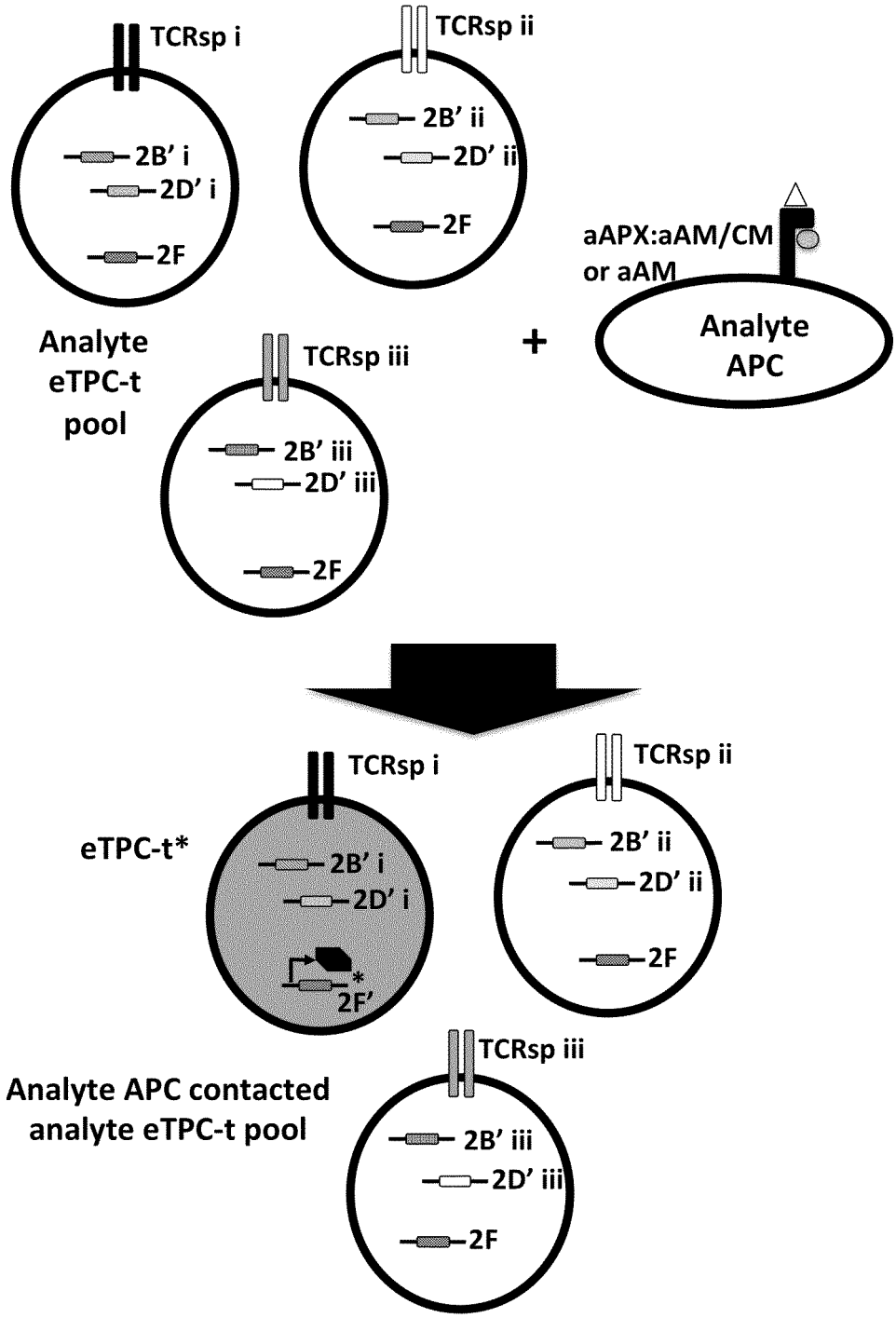

TCRsp i
2B' i
2D' i
2F
TCRsp ii
2B' ii
2D' ii
2F
aAPX:aAM/CM
or aAM
Analyte
APC
+
Analyte
eTPC-t
pool
TCRsp iii
2B' iii
2D' iii
2F
TCRsp i
eTPC-t*
2B' i
2D' i
2F'
TCRsp ii
2B' ii
2D' ii
2F
TCRsp iii
2B' iii
2D' iii
2F
Analyte APC contacted
analyte eTPC-t pool

Figure 22

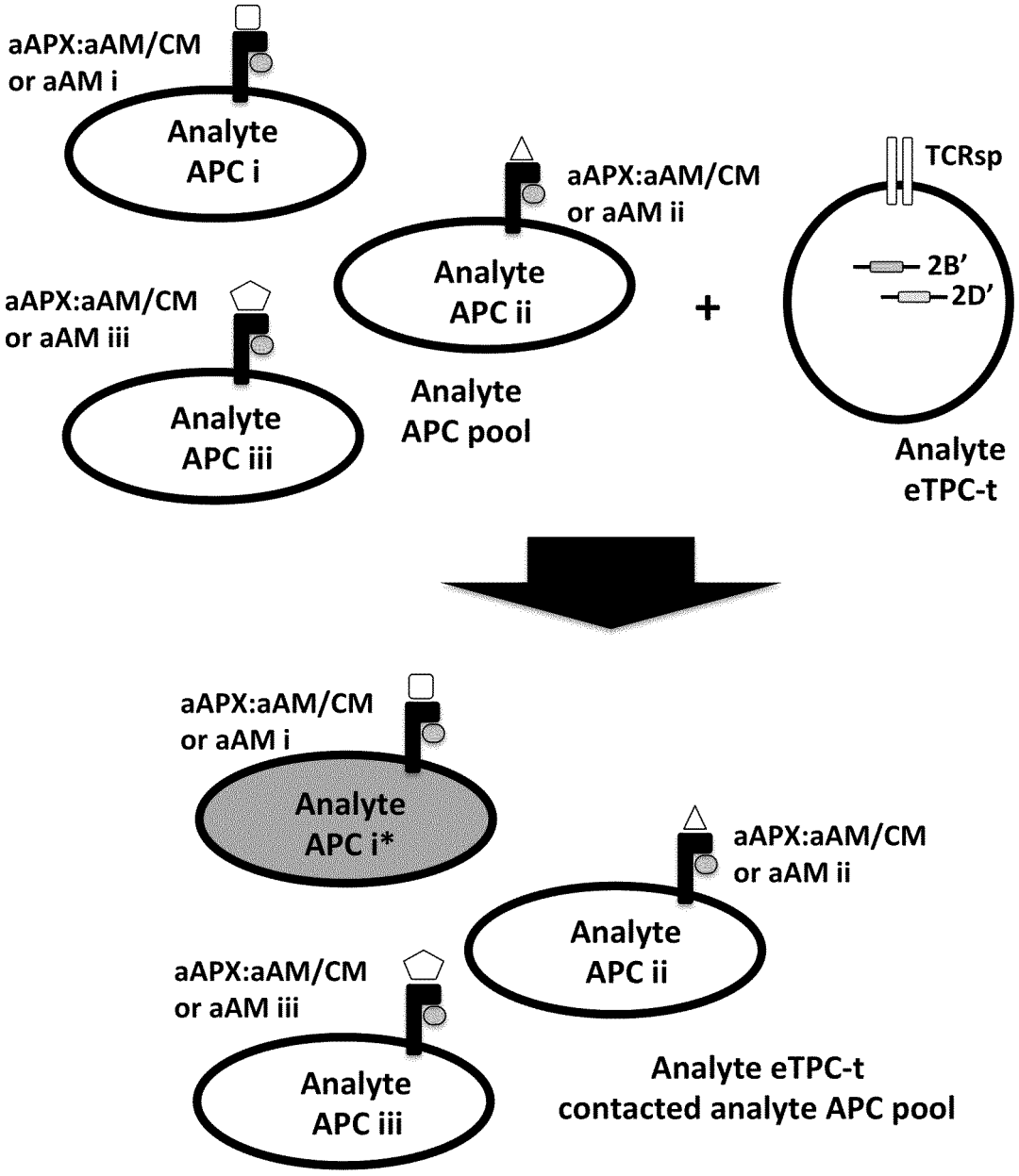

aAPX:aAM/CM
or aAM i
Analyte
APC i
aAPX:aAM/CM
or aAM ii
Analyte
APC ii
aAPX:aAM/CM
or aAM iii
Analyte
APC iii
Analyte
APC pool
+
TCRsp
2B'
2D'
Analyte
eTPC-t
aAPX:aAM/CM
or aAM i
Analyte
APC i*
aAPX:aAM/CM
or aAM ii
Analyte
APC ii
aAPX:aAM/CM
or aAM iii
Analyte
APC iii
Analyte eTPC-t
contacted analyte APC pool

Figure 23

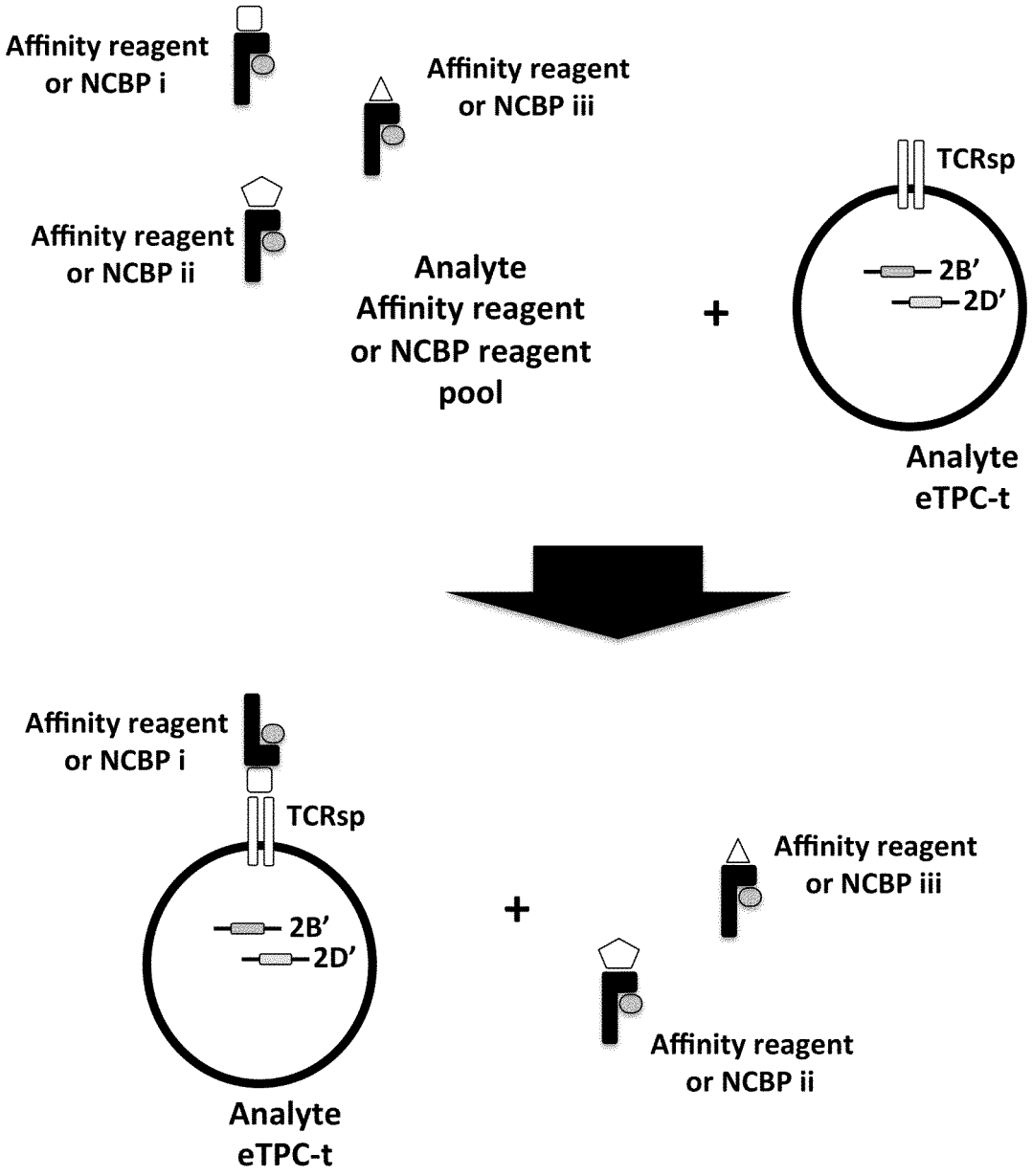

Affinity reagent or NCBP i
Affinity reagent or NCBP iii
Affinity reagent or NCBP ii
Analyte Affinity reagent or NCBP reagent pool
+
TCRsp
2B'
2D'
Analyte eTPC-t
Affinity reagent or NCBP i
TCRsp
2B'
2D'
Analyte eTPC-t
+
Affinity reagent or NCBP iii
Affinity reagent or NCBP ii

Figure 24

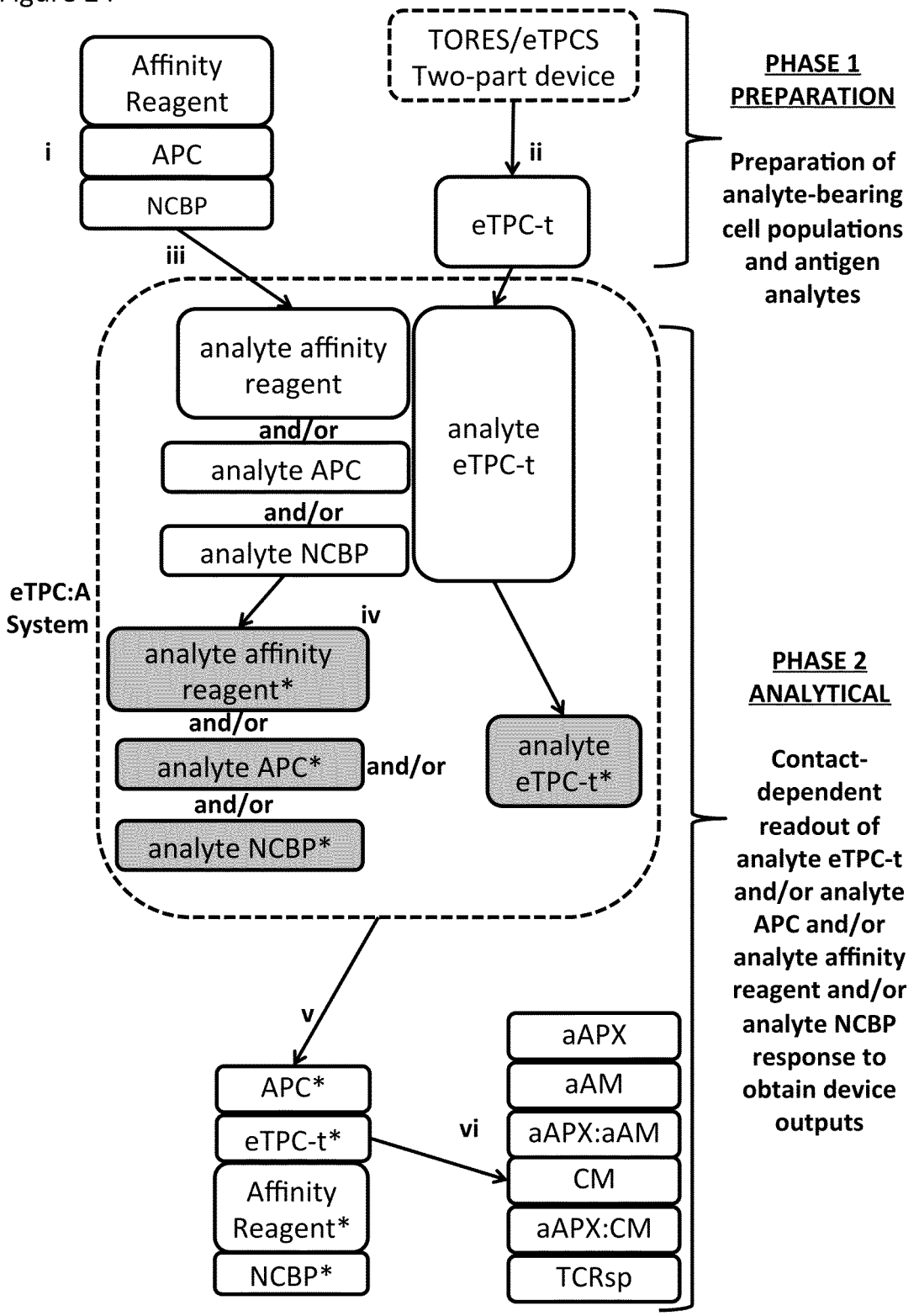
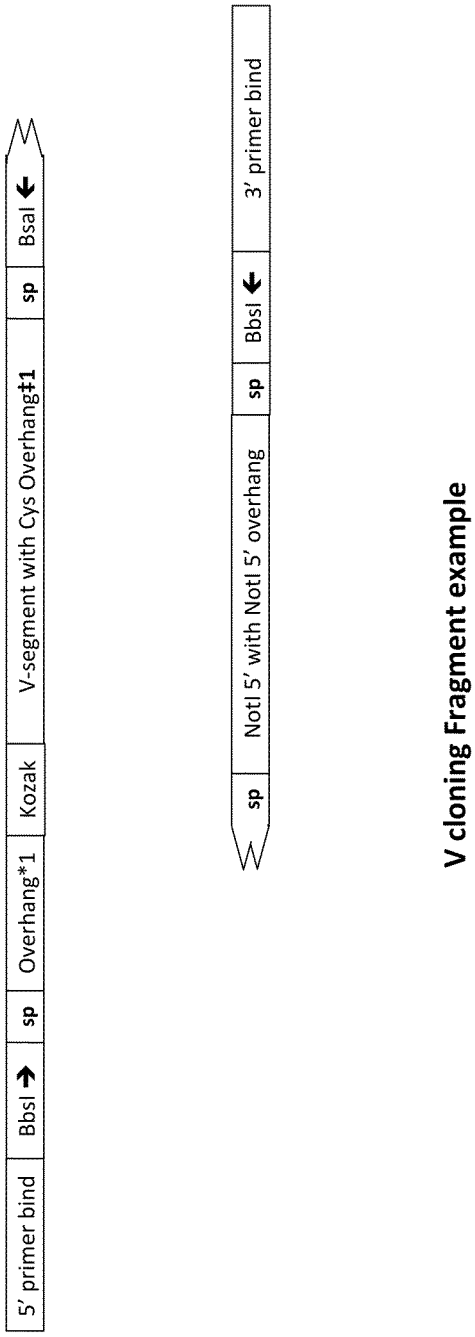
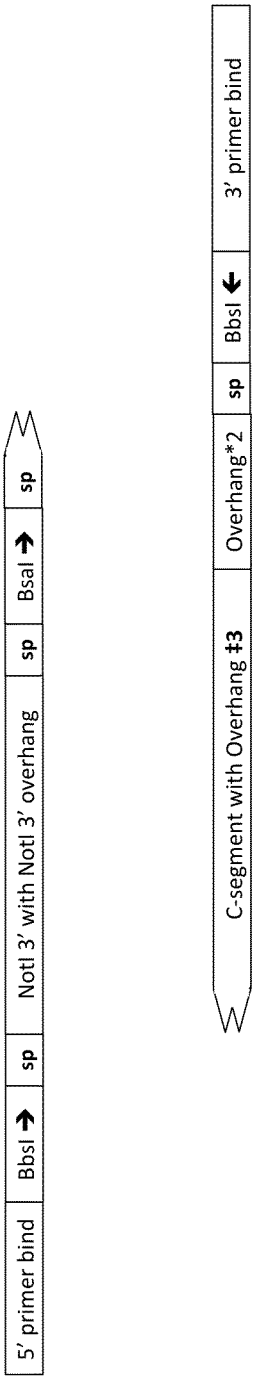

Affinity Reagent
APC
NCBP
i
iii
TORES/eTPCS Two-part device
ii
eTPC-t
PHASE 1 PREPARATION
Preparation of analyte-bearing cell populations and antigen analytes
analyte affinity reagent
and/or
analyte APC
and/or
analyte NCBP
analyte eTPC-t
eTPC:A System
iv
analyte affinity reagent*
and/or
analyte APC*
and/or
analyte NCBP*
and/or
analyte eTPC-t*
PHASE 2 ANALYTICAL
Contact-dependent readout of analyte eTPC-t and/or analyte APC and/or analyte affinity reagent and/or analyte NCBP response to obtain device outputs
v
APC*
eTPC-t*
Affinity Reagent*
NCBP*
vi
aAPX
aAM
aAPX:aAM
CM
aAPX:CM
TCRsp

Figure 25



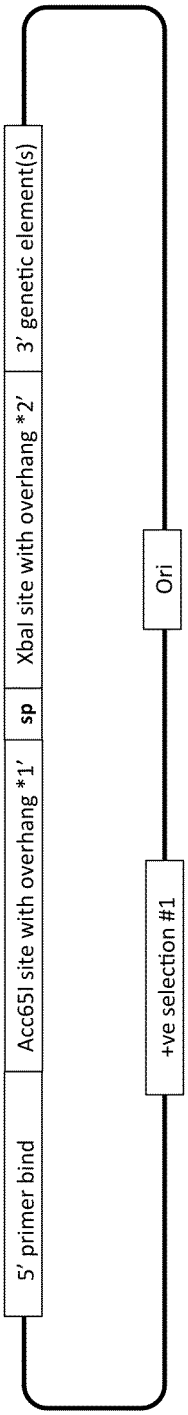
V cloning Fragment example

Figure 26



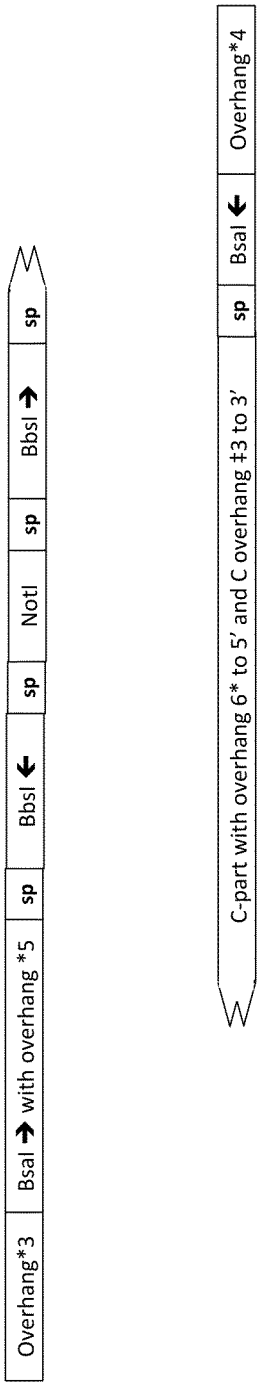
C cloning Fragment example

Figure 27



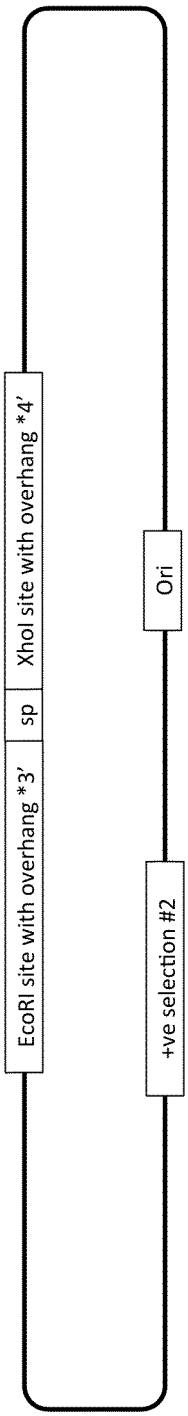
V-C entry vector backbone example

Figure 28



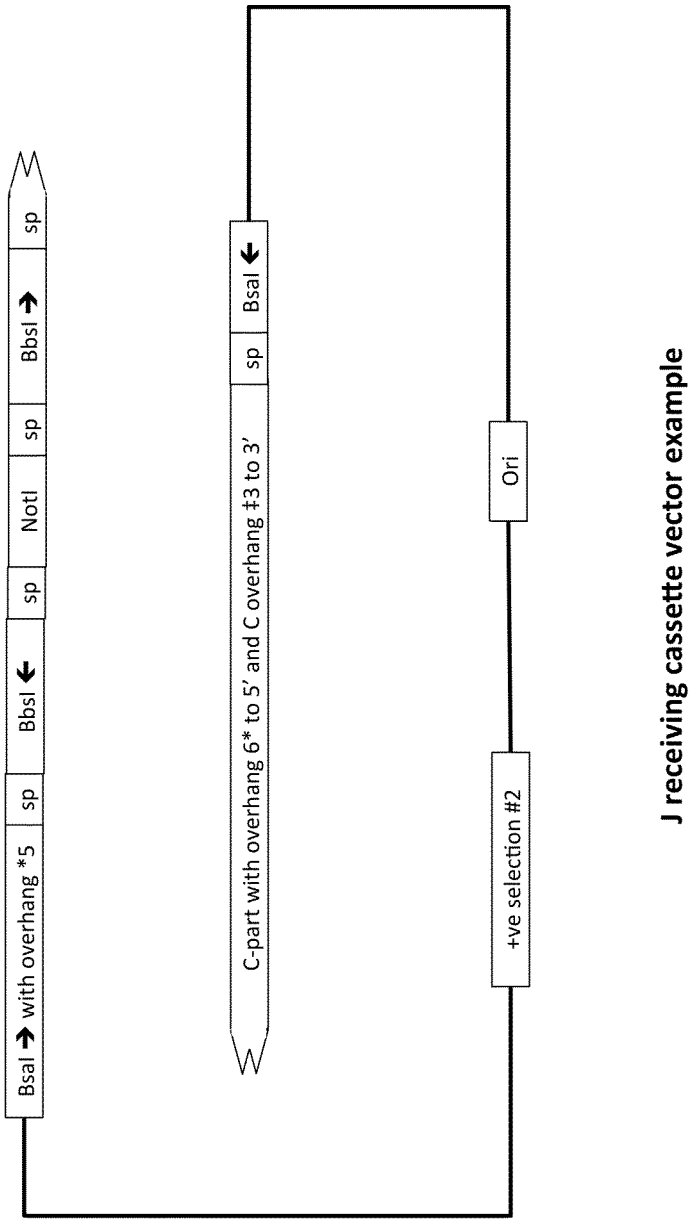
J receiving cassette fragment example

Figure 29



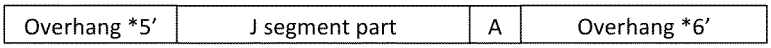
J Donor vector backbone example

Figure 30



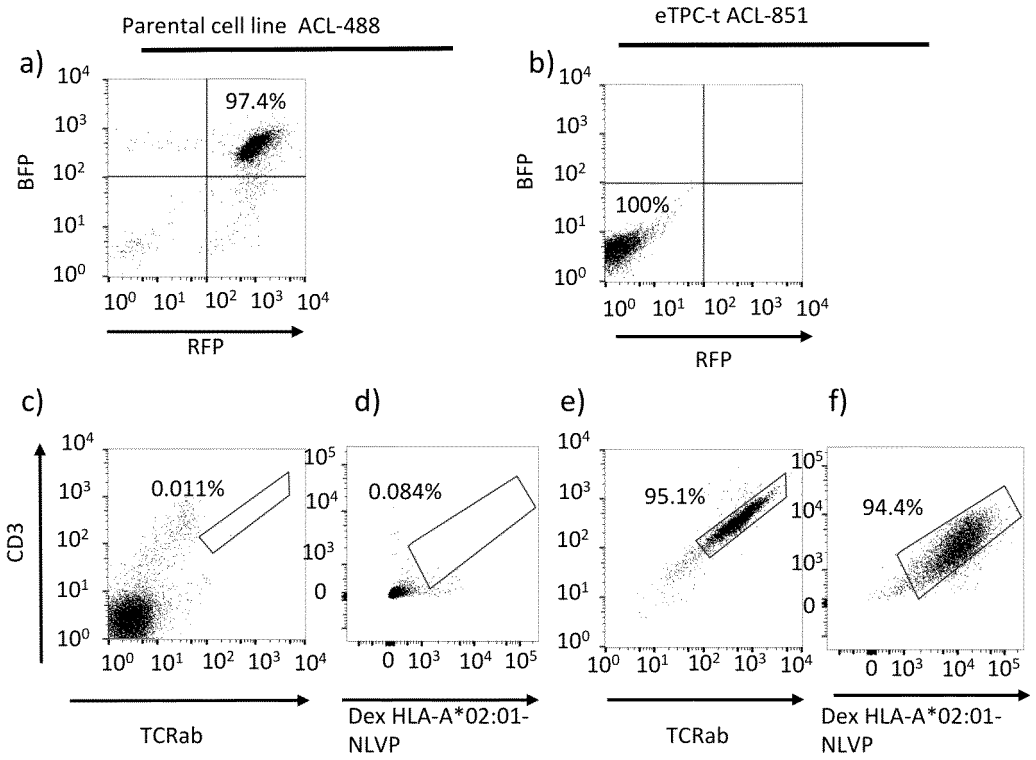
J receiving cassette vector example

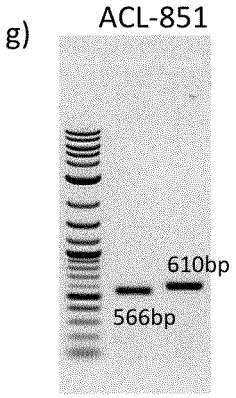
Figure 31



J segment part example

Figure 32





h)

Copy number of integration DNA	
Observed Ratio TRAC/TCRalpha	Observed Ratio TRAC/TCRbeta
0,319	0,322

TCRa expected size:566 bp

TCRb expected size:610 bp

Figure 33

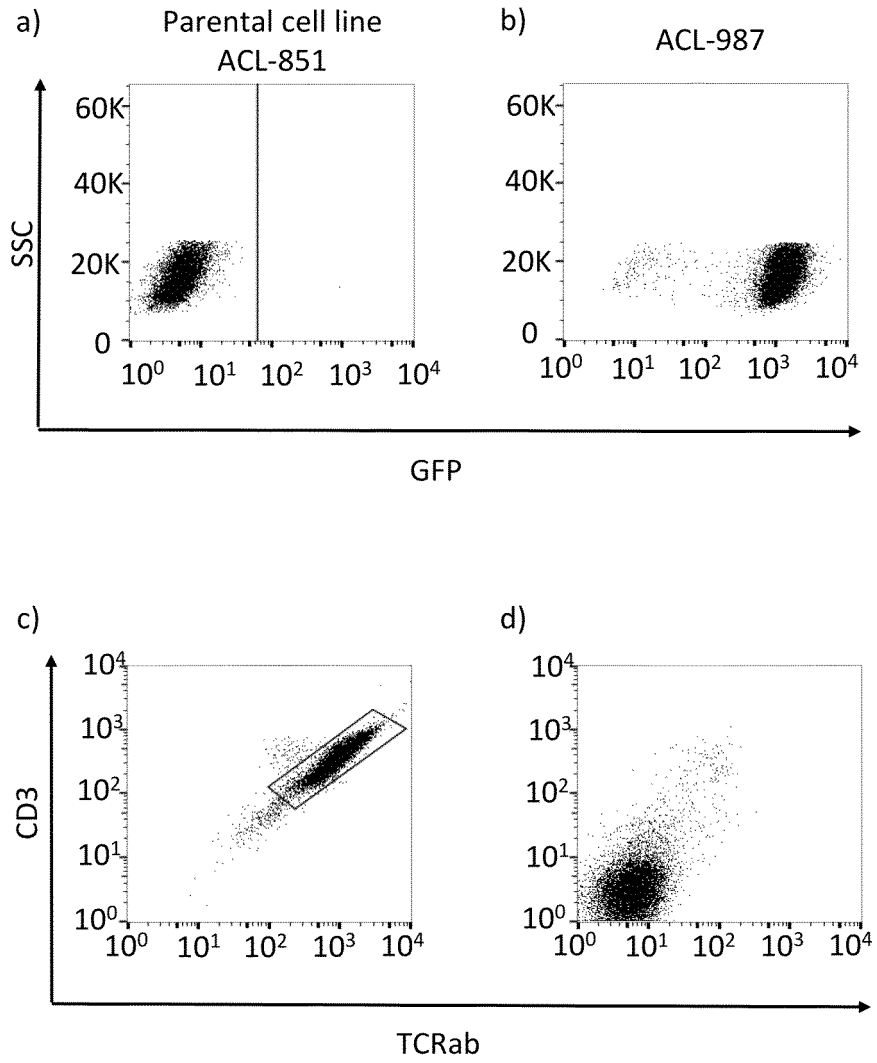

a)
Parental cell line
ACL-851
b)
ACL-987
60K
40K
20K
0
SSC
10^0 10^1 10^2 10^3 10^4
GFP
c)
d)
CD3
TCRab

Figure 34

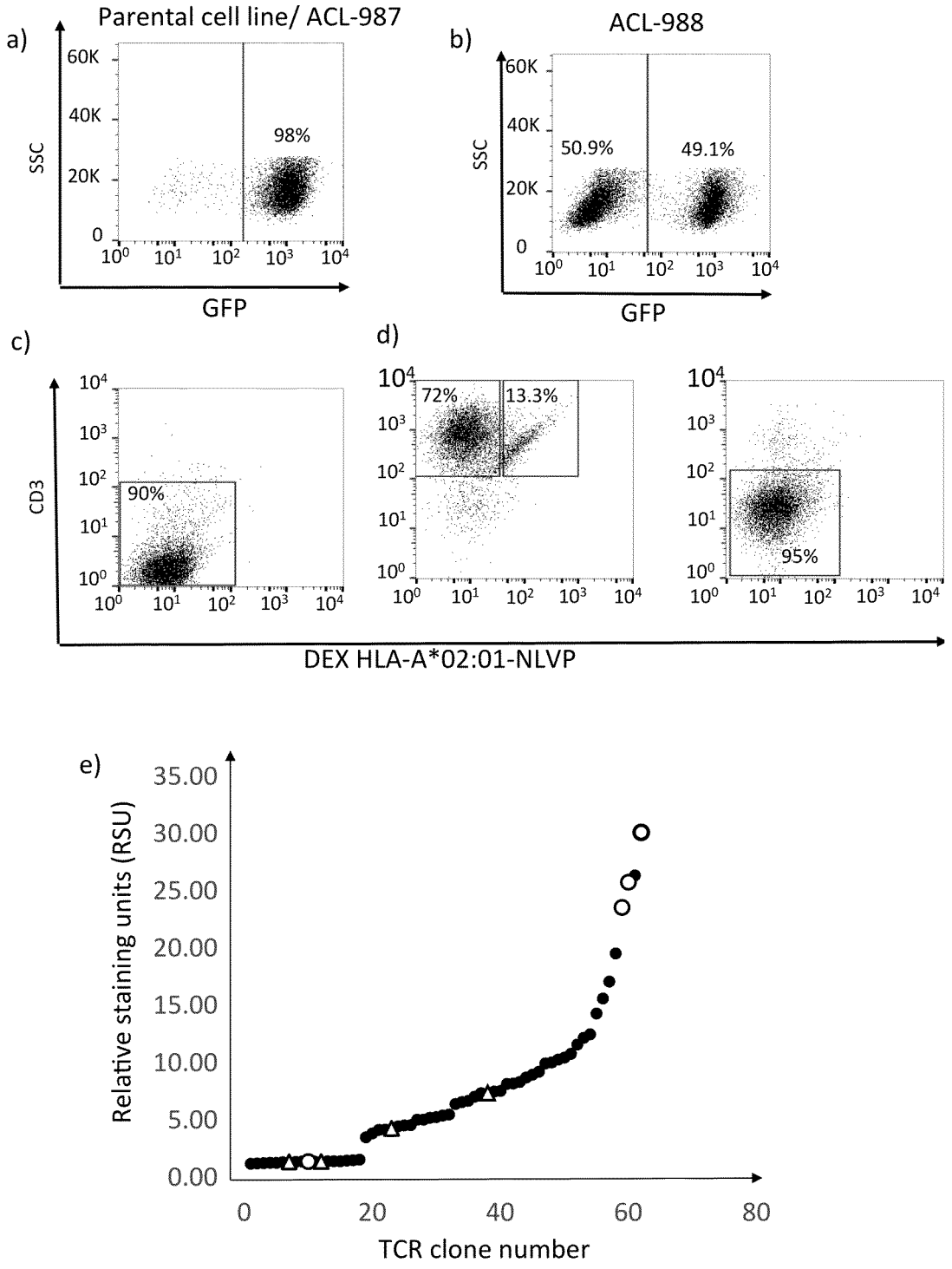
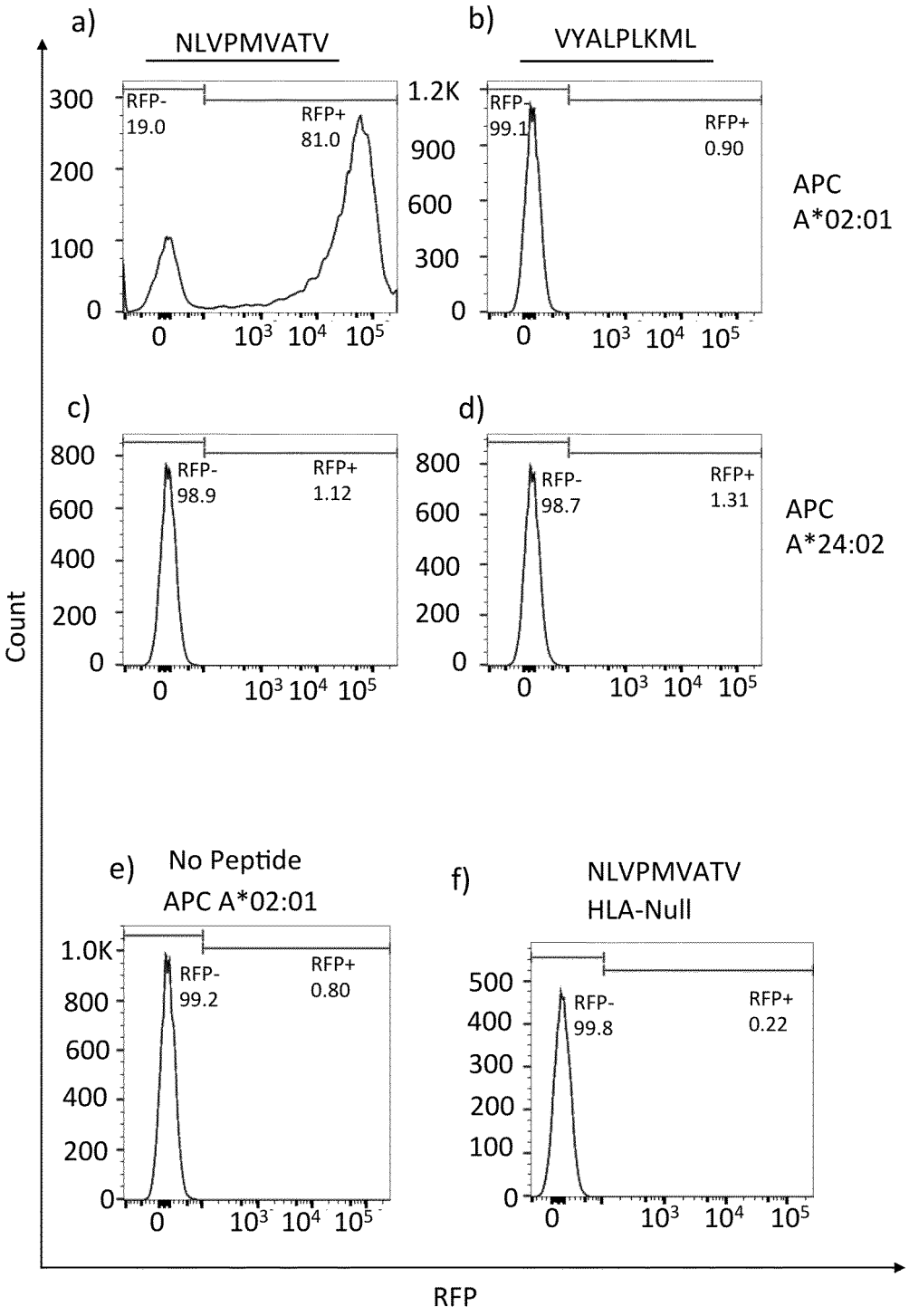

a)
Parental cell line/ ACL-987
60K
40K
20K
0
SSC
98%
GFP
b)
ACL-988
50.9%
49.1%
c)
CD3
90%
d)
72%
13.3%
95%
DEX HLA-A*02:01-NLVP
e)
Relative staining units (RSU)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0
20
40
60
80
TCR clone number

Figure 35


a)
NLVPMVATV
RFP-
19.0
RFP+
81.0
b)
VYALPLKML
RFP-
99.1
RFP+
0.90
APC
A*02:01
c)
RFP-
98.9
RFP+
1.12
d)
RFP-
98.7
RFP+
1.31
APC
A*24:02
Count
e)
No Peptide
APC A*02:01
RFP-
99.2
RFP+
0.80
f)
NLVPMVATV
HLA-Null
RFP-
99.8
RFP+
0.22
RFP