Figure 1

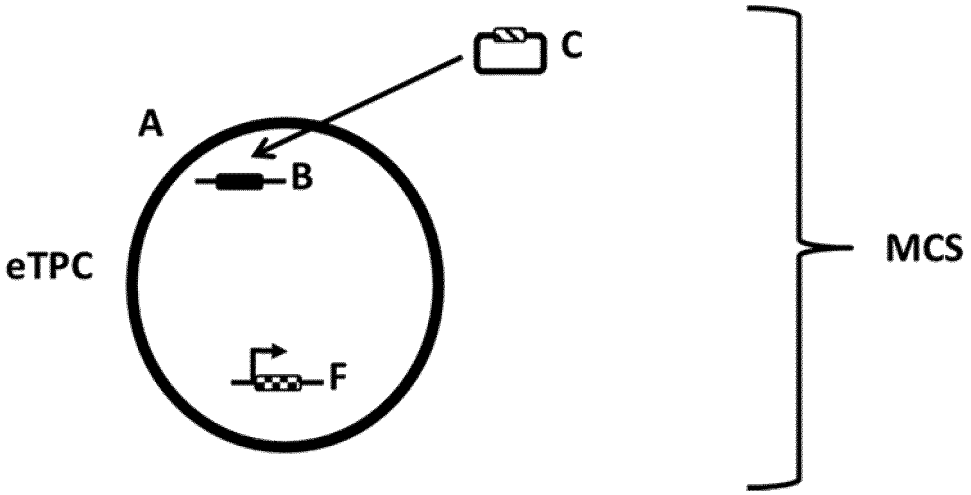


Figure 2

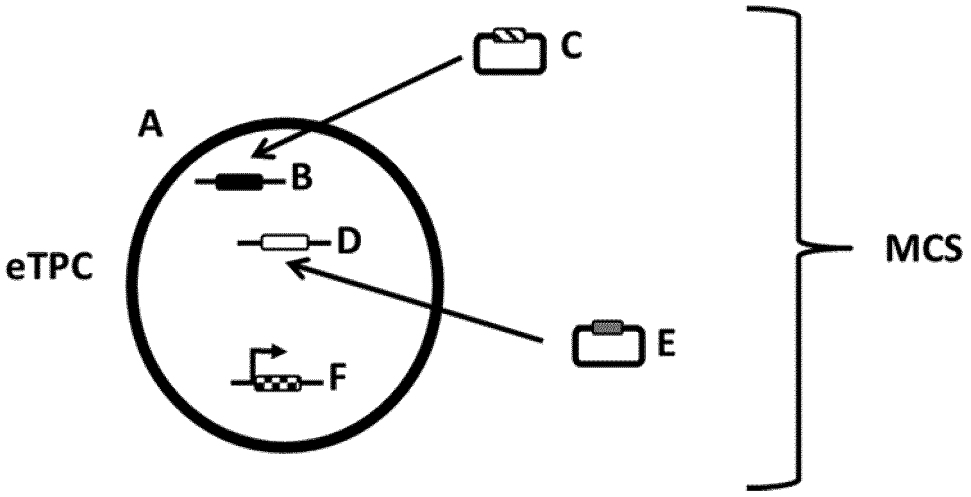


Figure 3

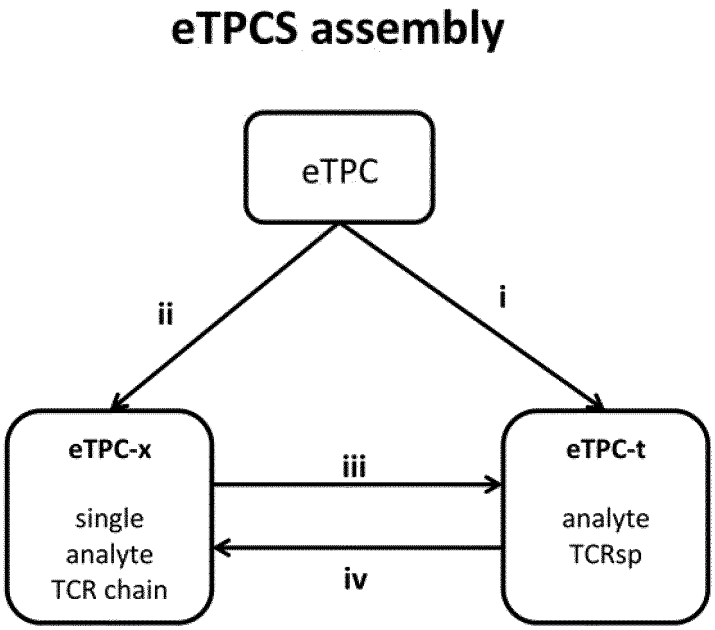

eTPCS assembly
eTPC
ii
i
eTPC-x
single
analyte
TCR chain
iii
iv
eTPC-t
analyte
TCRsp

Figure 4

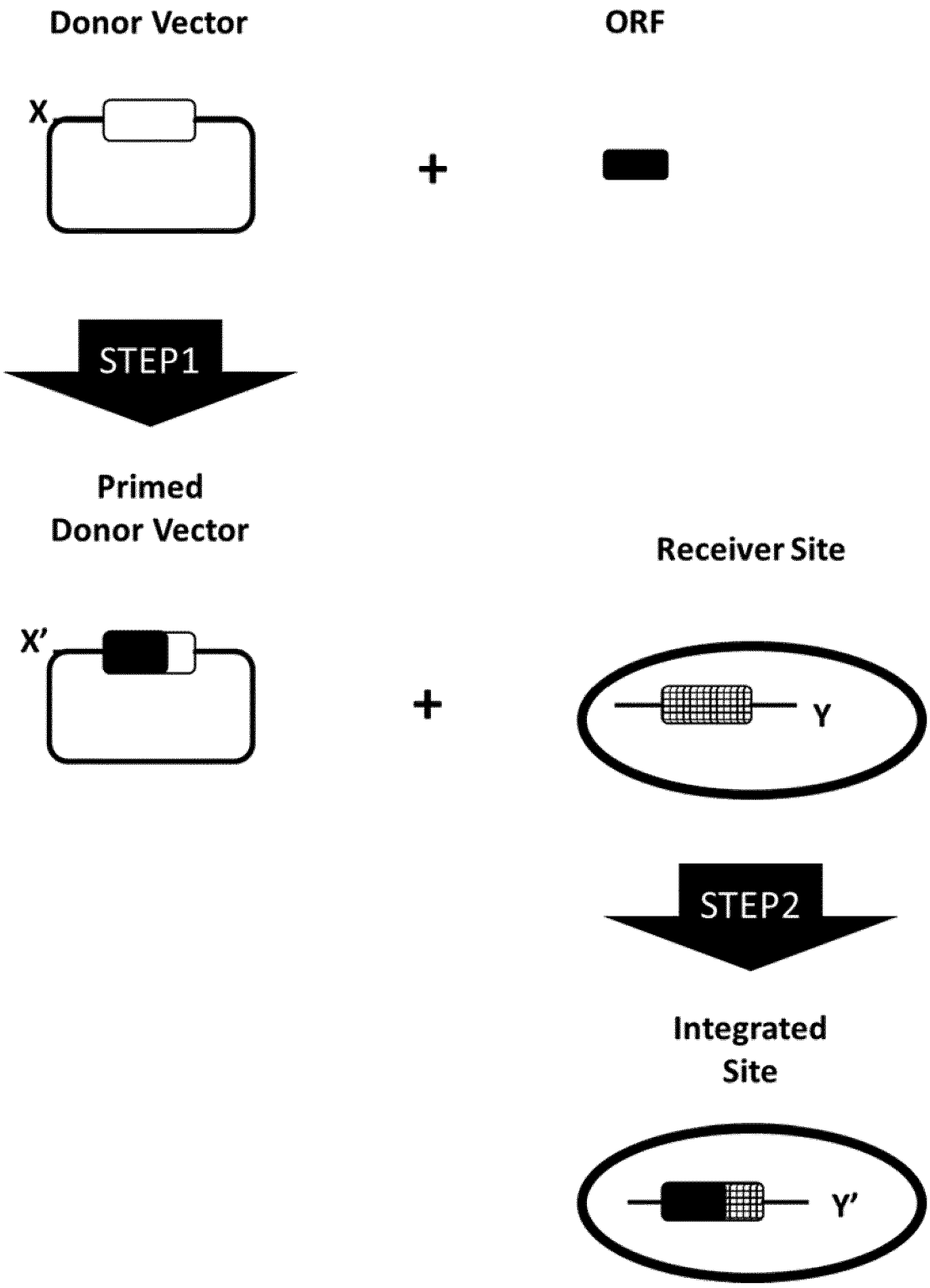

Donor Vector
ORF
X
+
STEP1
Primed
Donor Vector
Receiver Site
X'
+
Y
STEP2
Integrated
Site
Y'

Figure 5

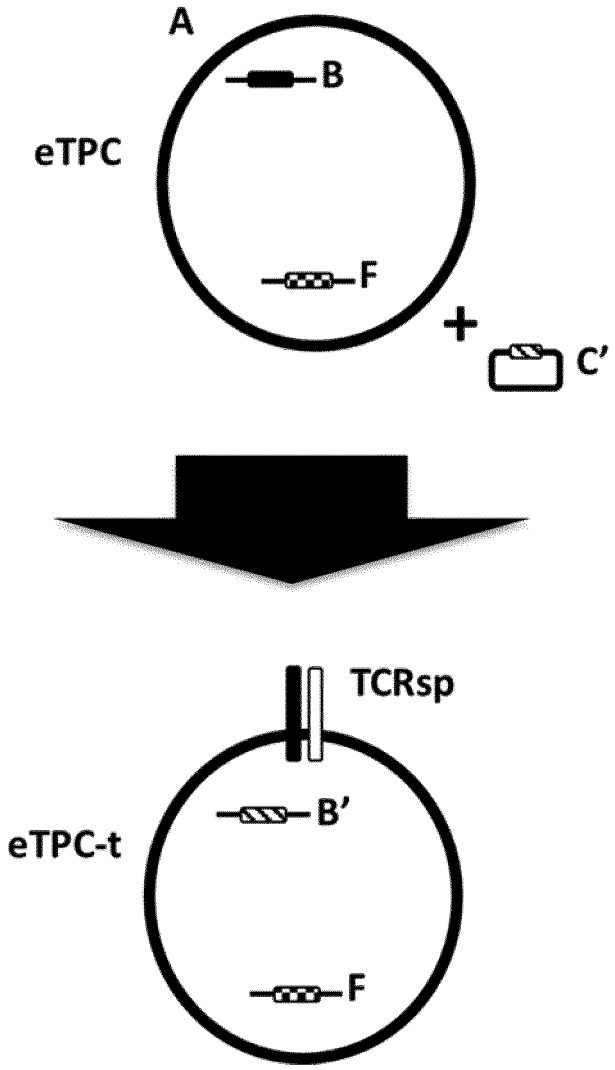


Figure 6

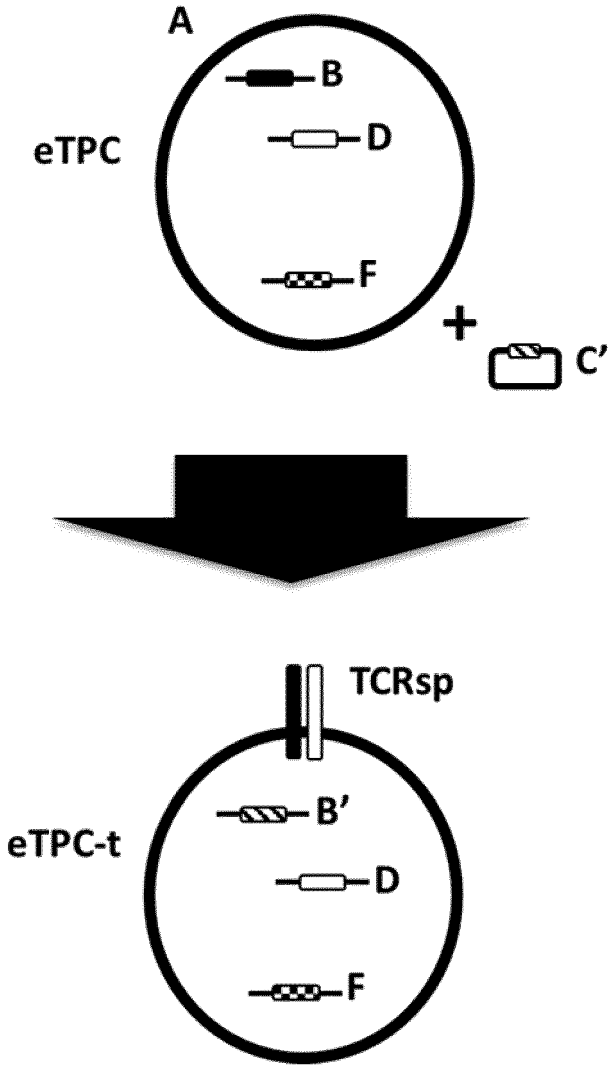


Figure 7

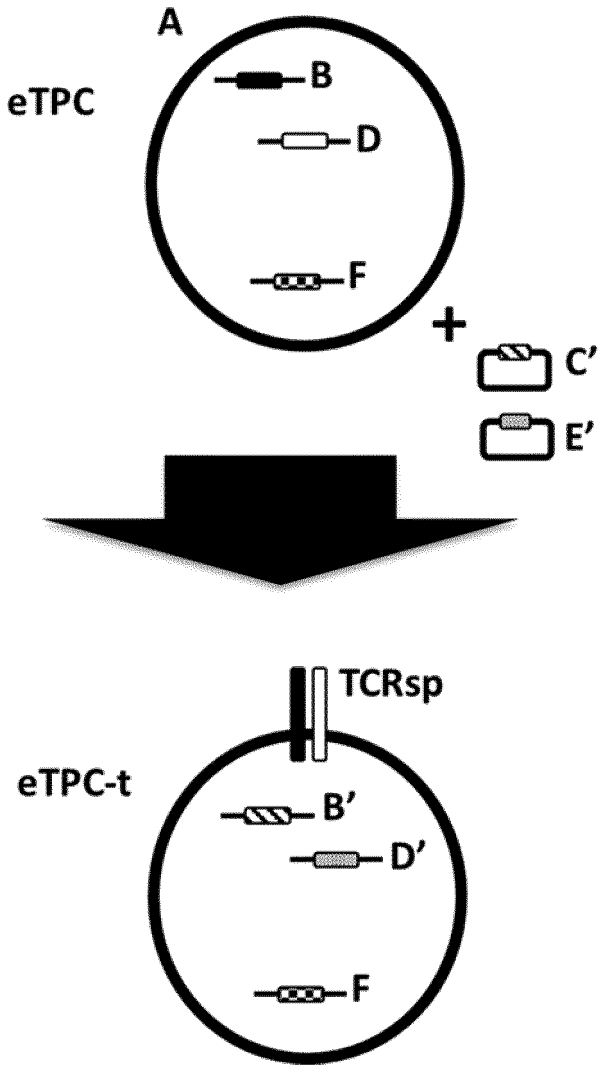


Figure 8

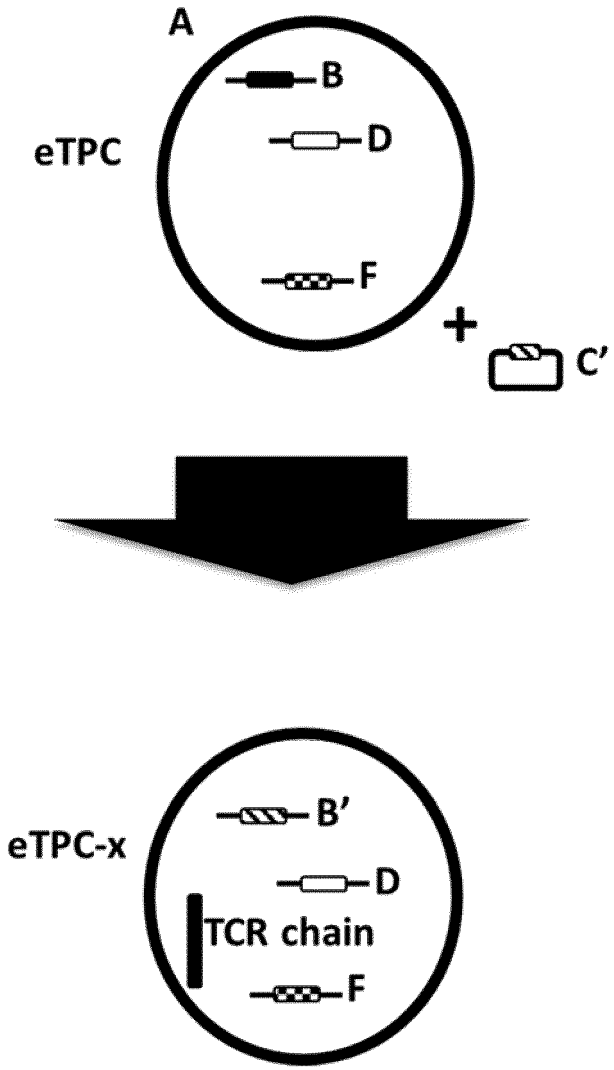


Figure 9

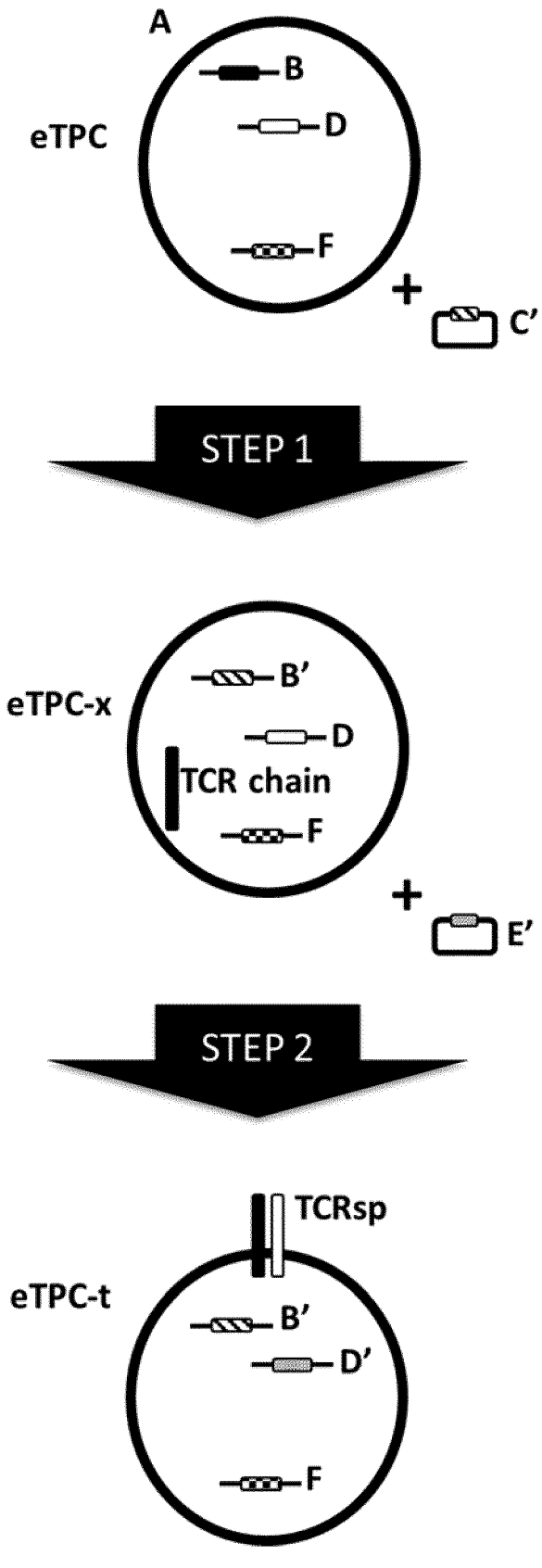


Figure 10

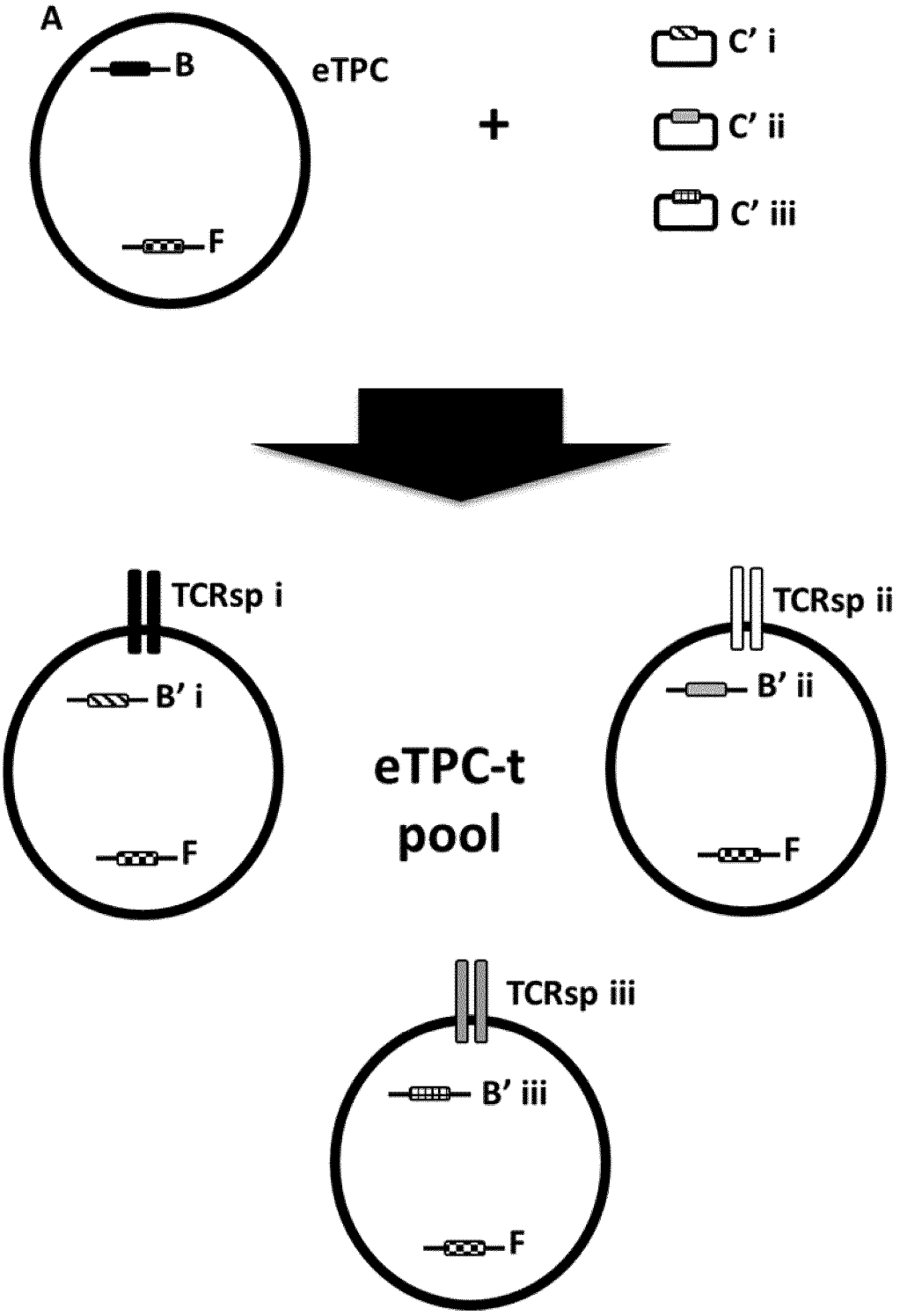

A
B
eTPC
F
+
C' i
C' ii
C' iii
TCRsp i
B' i
F
eTPC-t
pool
TCRsp ii
B' ii
F
TCRsp iii
B' iii
F

Figure 11

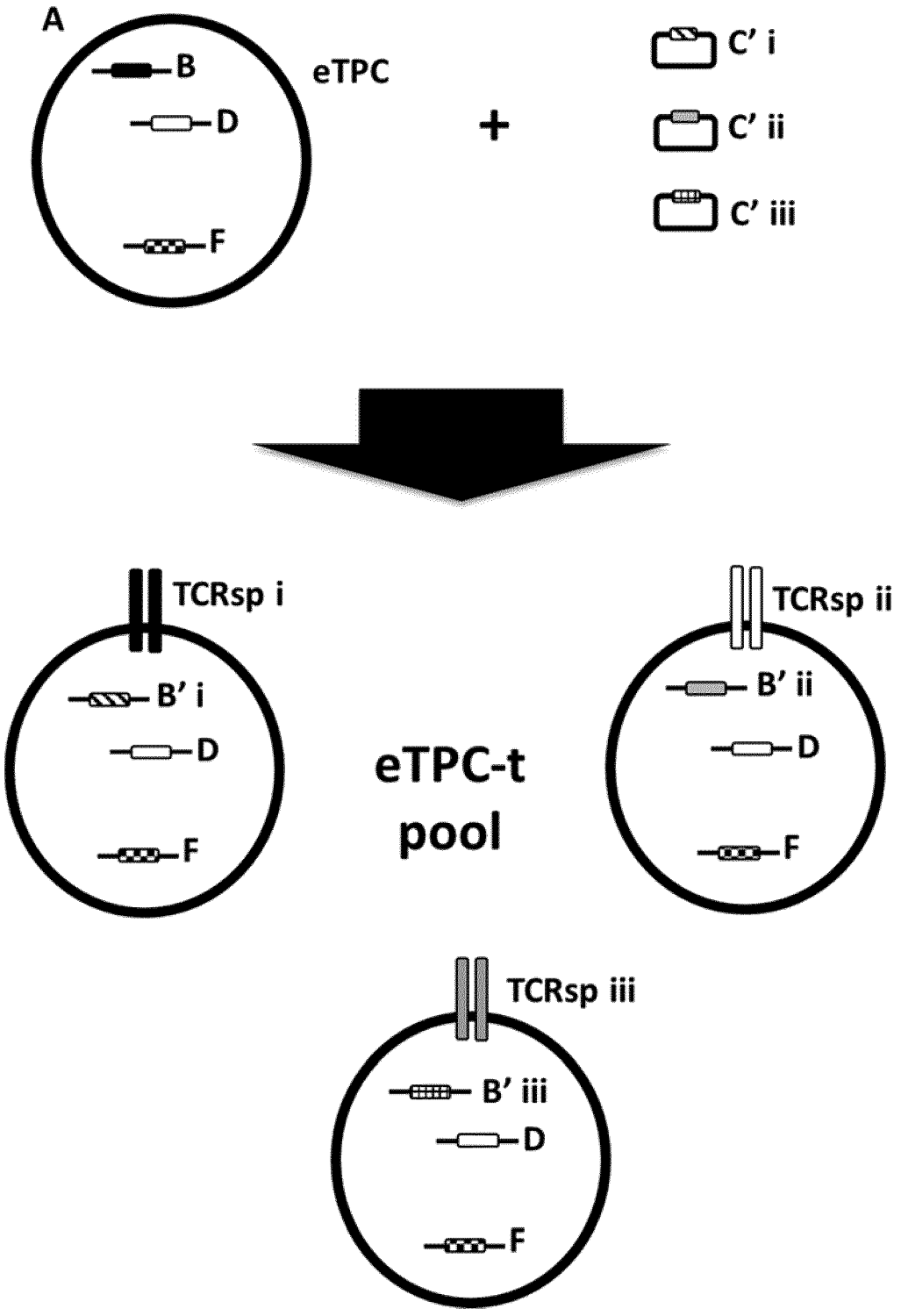

A
B
D
F
eTPC
+
C' i
C' ii
C' iii
TCRsp i
B' i
D
F
eTPC-t
pool
TCRsp ii
B' ii
D
F
TCRsp iii
B' iii
D
F

Figure 12

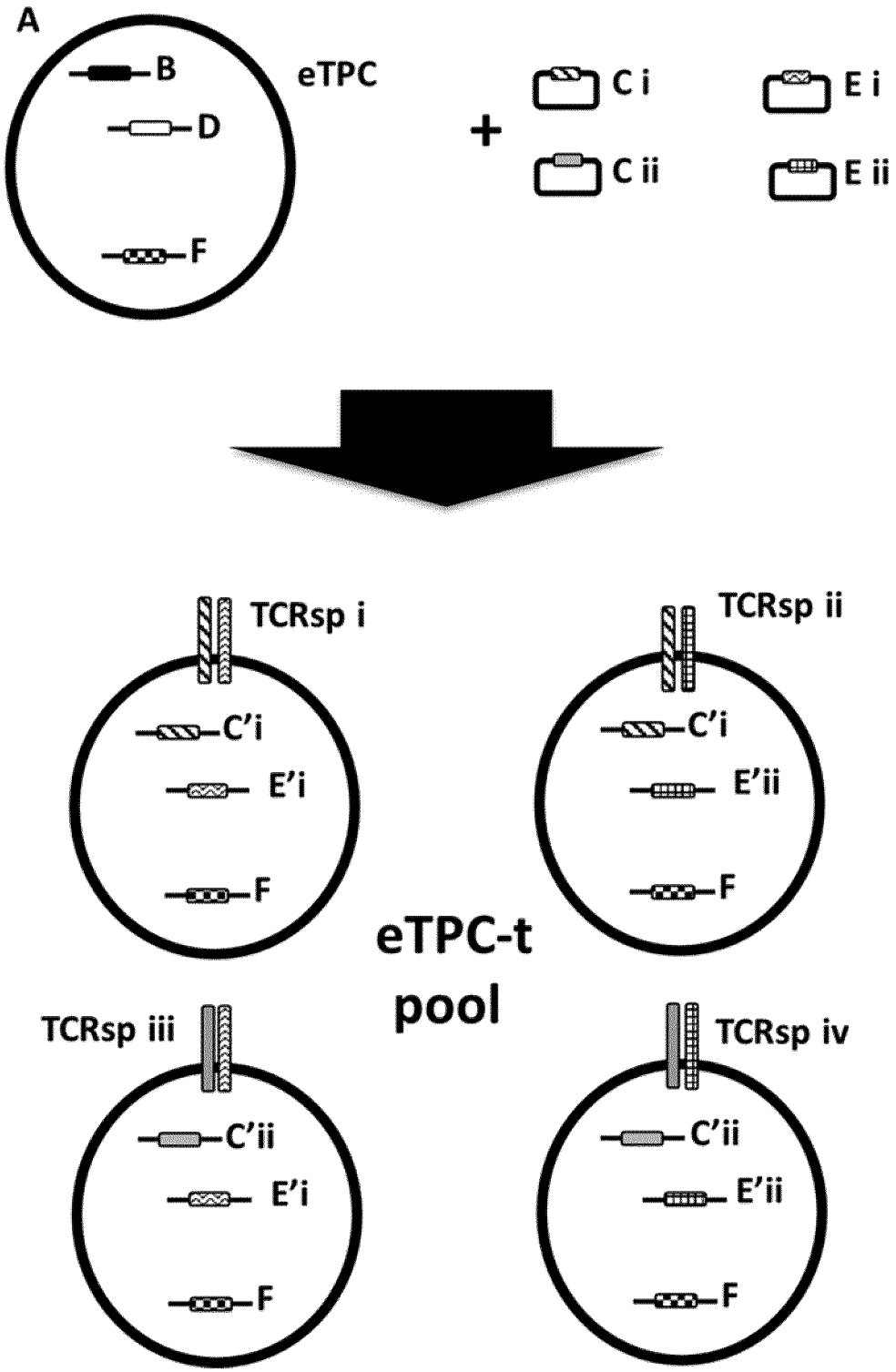

A
B
D
F
eTPC
+
C i
C ii
E i
E ii
TCRsp i
C'i
E'i
F
TCRsp ii
C'i
E'ii
F
eTPC-t
pool
TCRsp iii
C'ii
E'i
F
TCRsp iv
C'ii
E'ii
F

Figure 13

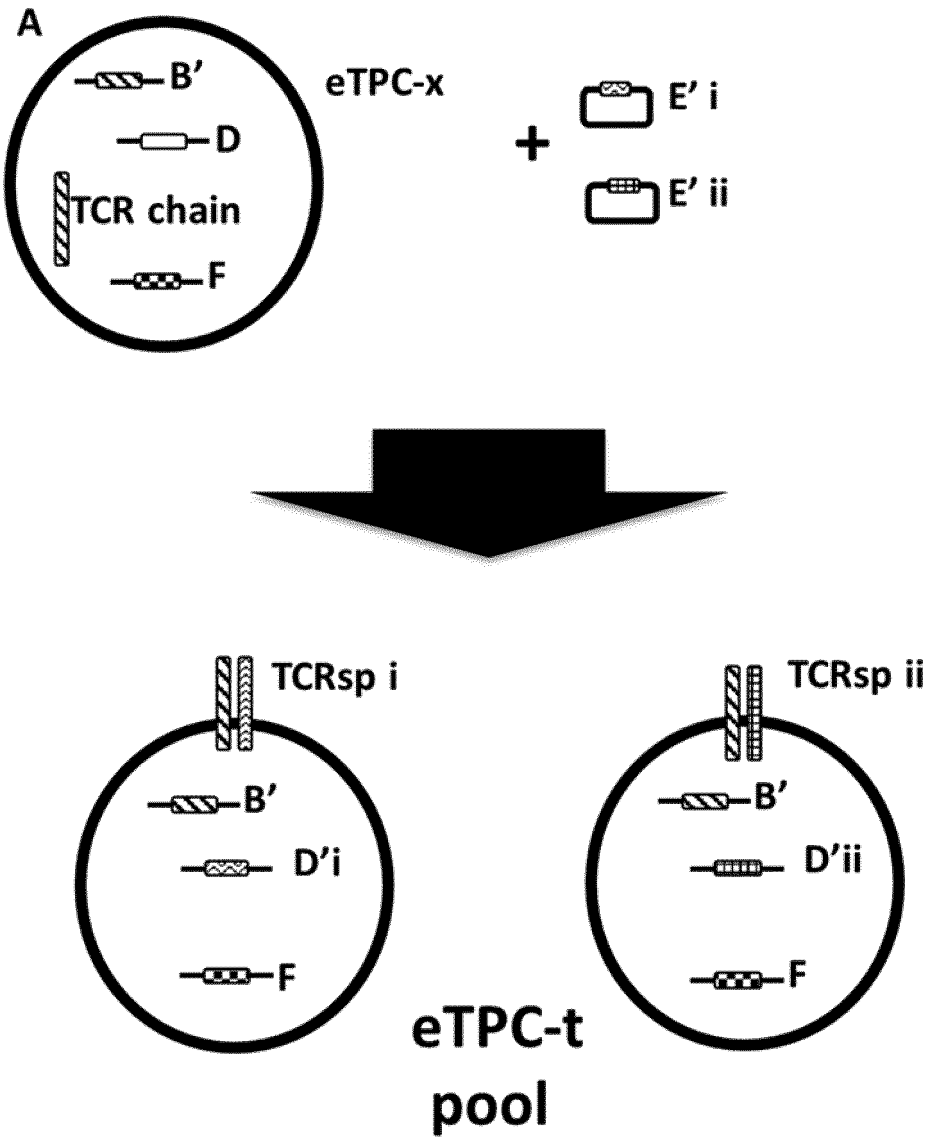

A
B'
D
TCR chain
F
eTPC-x
+
E' i
E' ii
TCRsp i
B'
D'i
F
TCRsp ii
B'
D'ii
F
eTPC-t pool

Figure 14

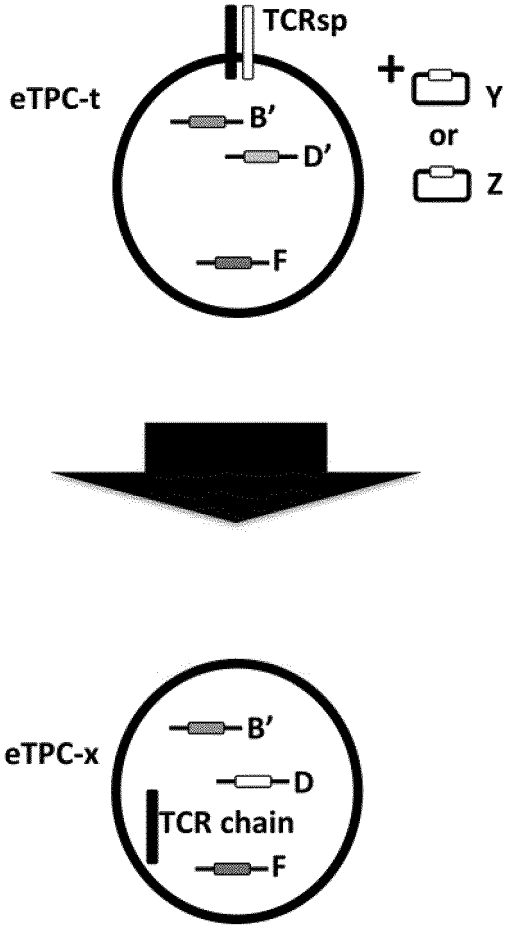


Figure 15

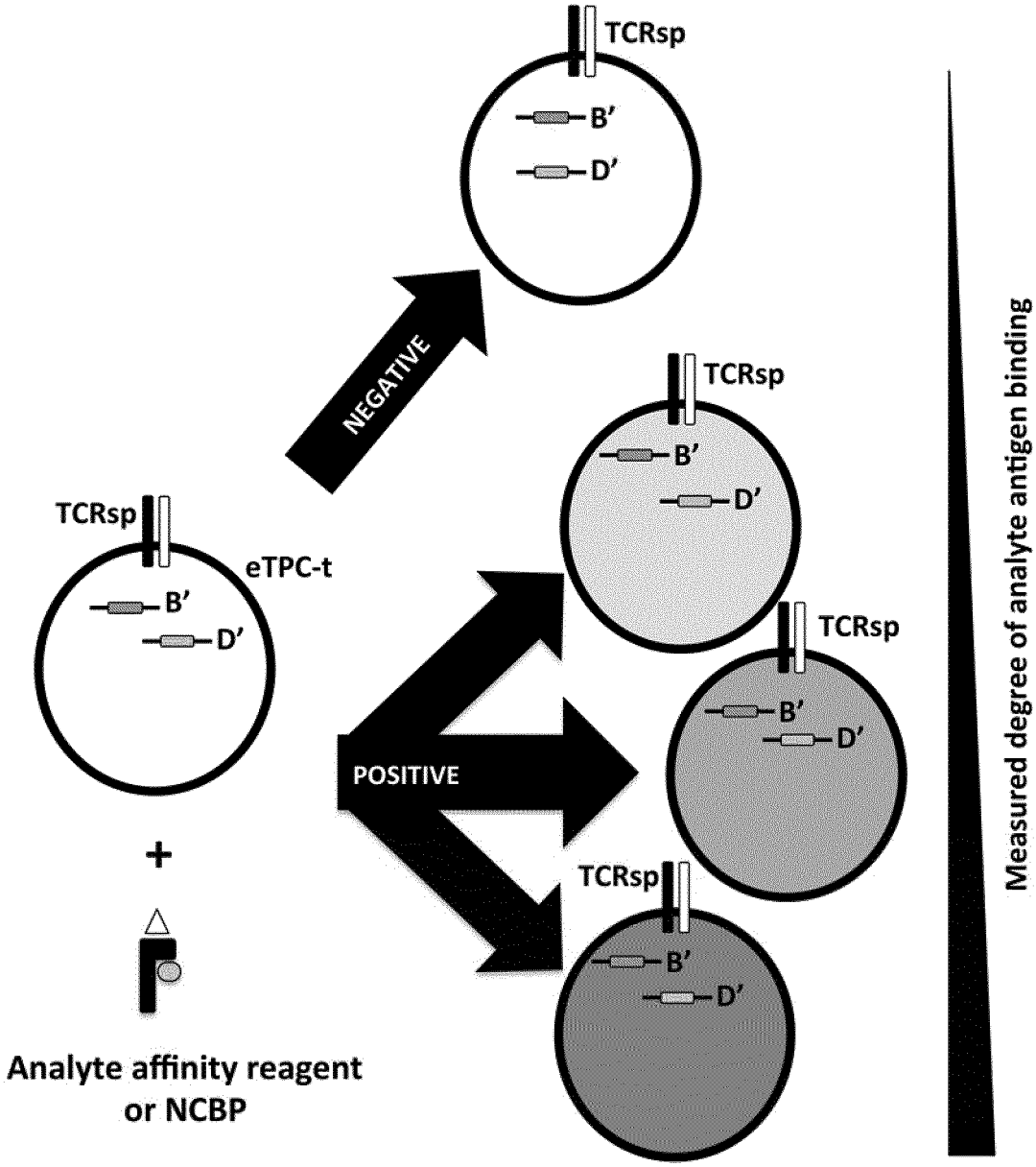

TCRsp
B′
D′
NEGATIVE
TCRsp
B′
D′
TCRsp
eTPC-t
B′
D′
POSITIVE
TCRsp
B′
D′
+
TCRsp
B′
D′
Analyte affinity reagent
or NCBP
Measured degree of analyte antigen binding

Figure 16

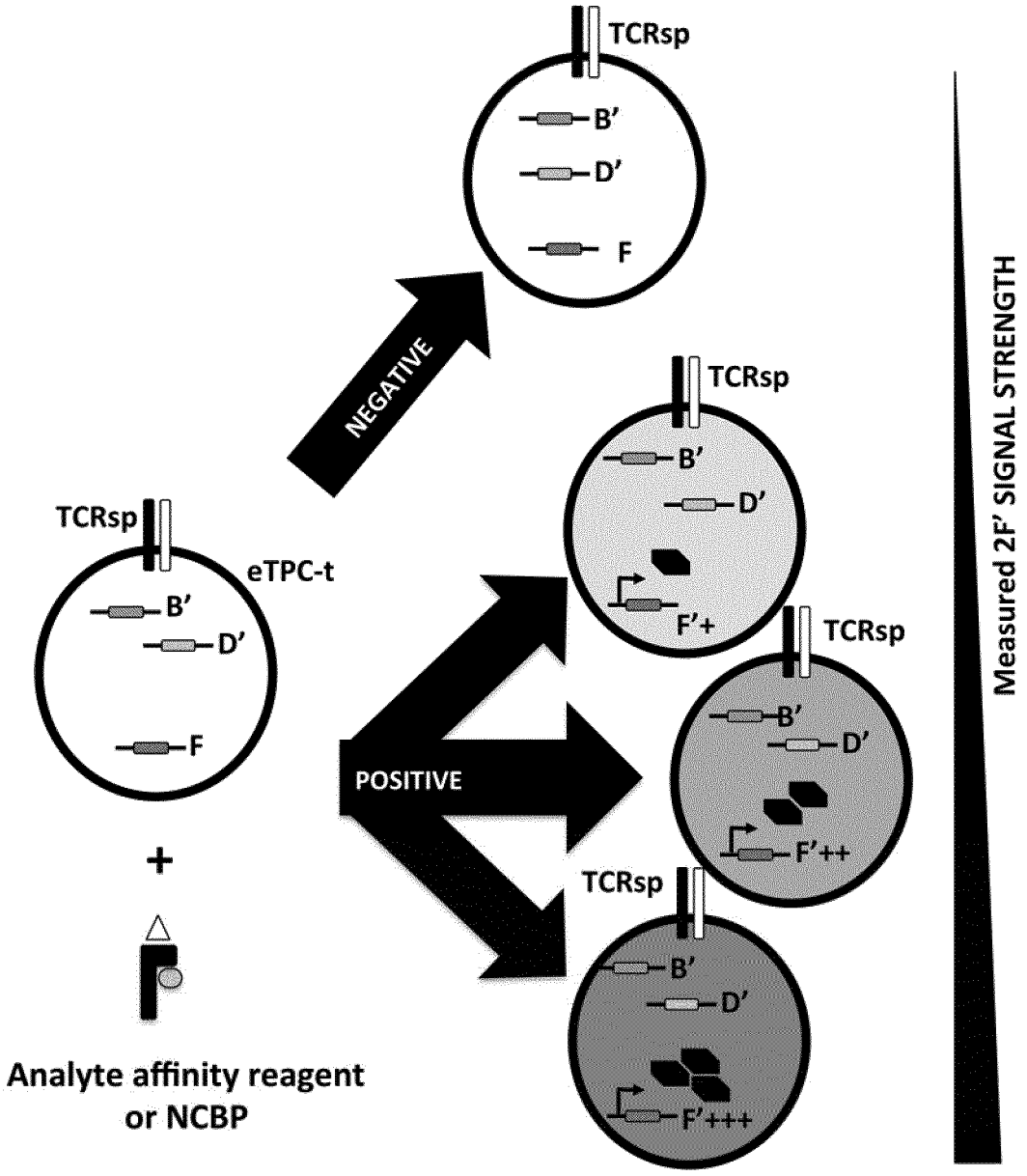

TCRsp
B'
D'
F
NEGATIVE
TCRsp
B'
D'
F'+
TCRsp
eTPC-t
B'
D'
F
POSITIVE
TCRsp
B'
D'
F'++
TCRsp
B'
D'
F'+++
+
Analyte affinity reagent
or NCBP
Measured 2F' SIGNAL STRENGTH

Figure 17

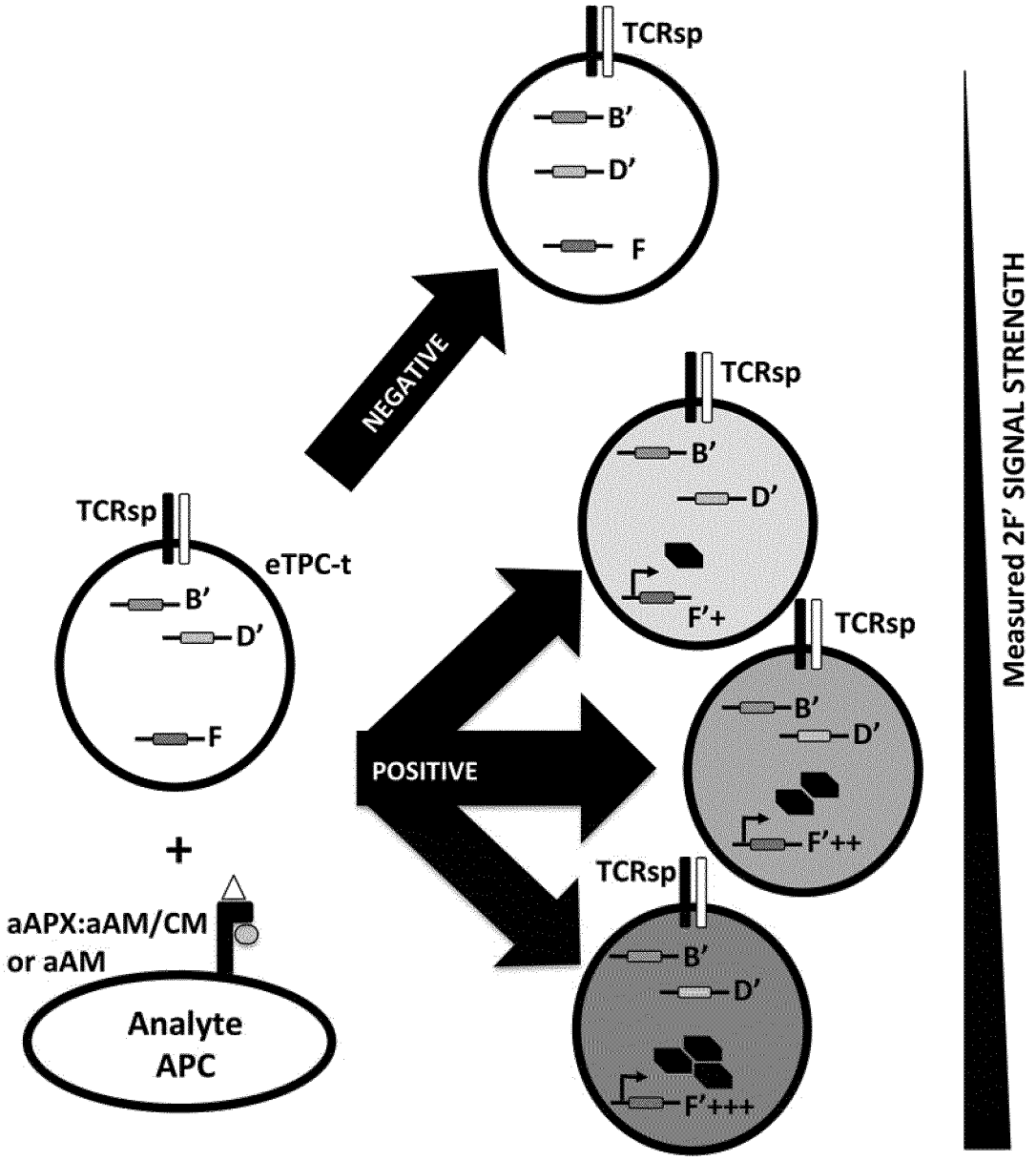

TCRsp
B'
D'
F
NEGATIVE
TCRsp
B'
D'
F'+
TCRsp
eTPC-t
B'
D'
F
POSITIVE
TCRsp
B'
D'
F'++
+
TCRsp
aAPX:aAM/CM
or aAM
B'
D'
Analyte
APC
F'+++
Measured 2F' SIGNAL STRENGTH

Figure 18

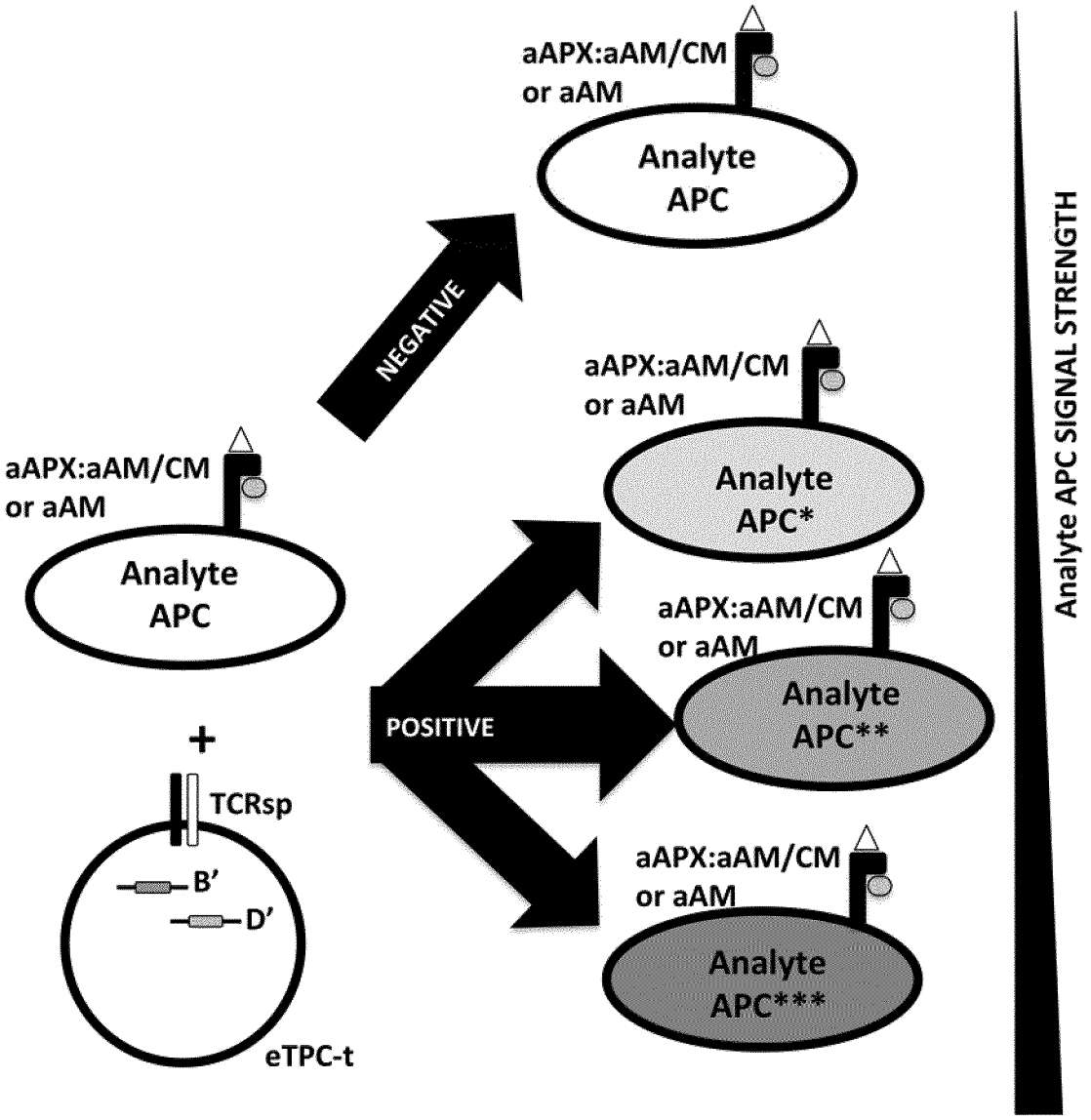

aAPX:aAM/CM
or aAM
Analyte
APC
NEGATIVE
aAPX:aAM/CM
or aAM
Analyte
APC*
aAPX:aAM/CM
or aAM
Analyte
APC
POSITIVE
aAPX:aAM/CM
or aAM
Analyte
APC**
+
TCRsp
B'
D'
aAPX:aAM/CM
or aAM
Analyte
APC***
eTPC-t
Analyte APC SIGNAL STRENGTH

Figure 19

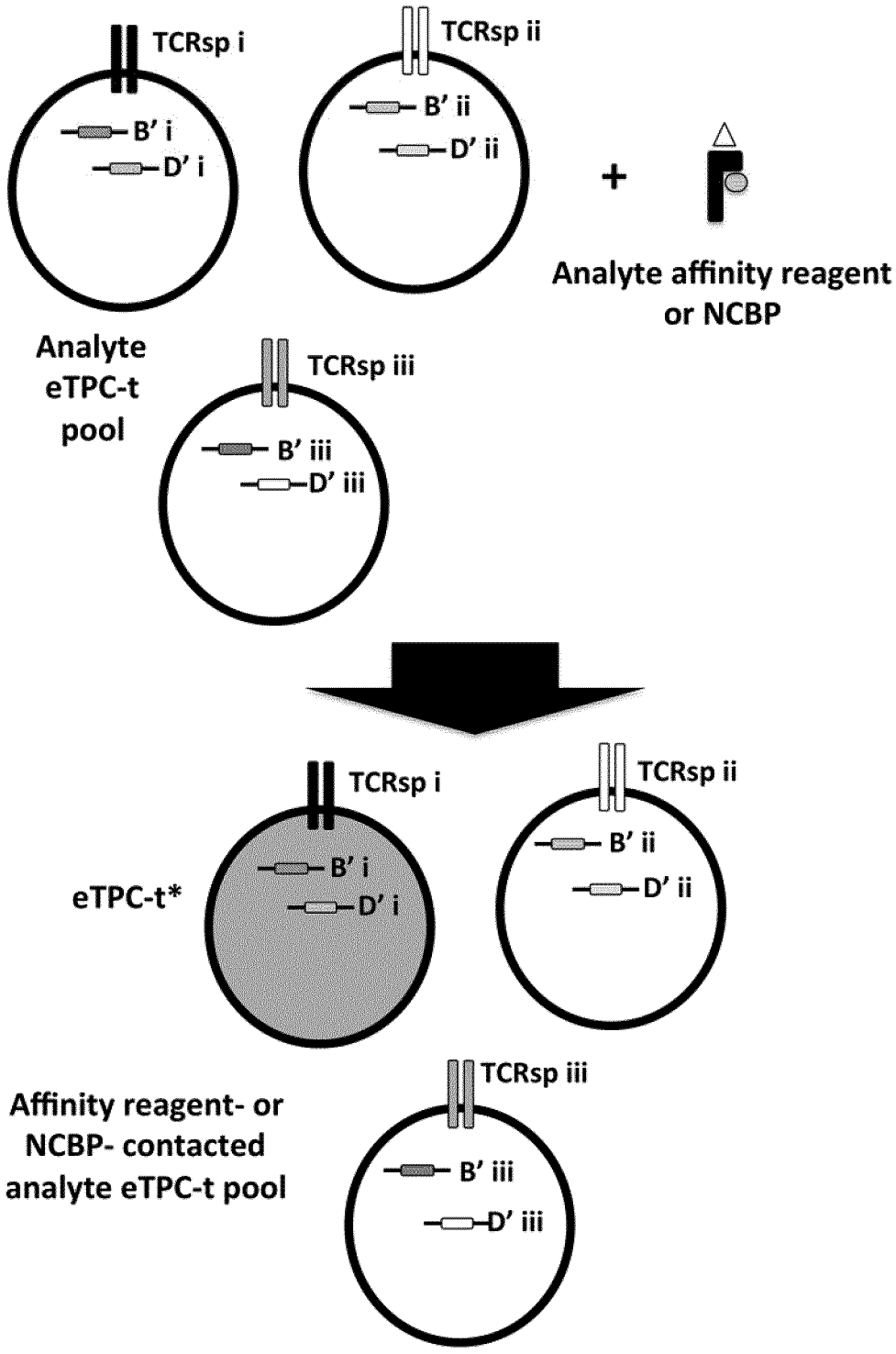

TCRsp i
B' i
D' i
TCRsp ii
B' ii
D' ii
+
Analyte affinity reagent or NCBP
Analyte eTPC-t pool
TCRsp iii
B' iii
D' iii
eTPC-t*
TCRsp i
B' i
D' i
TCRsp ii
B' ii
D' ii
Affinity reagent- or NCBP- contacted analyte eTPC-t pool
TCRsp iii
B' iii
D' iii

Figure 20

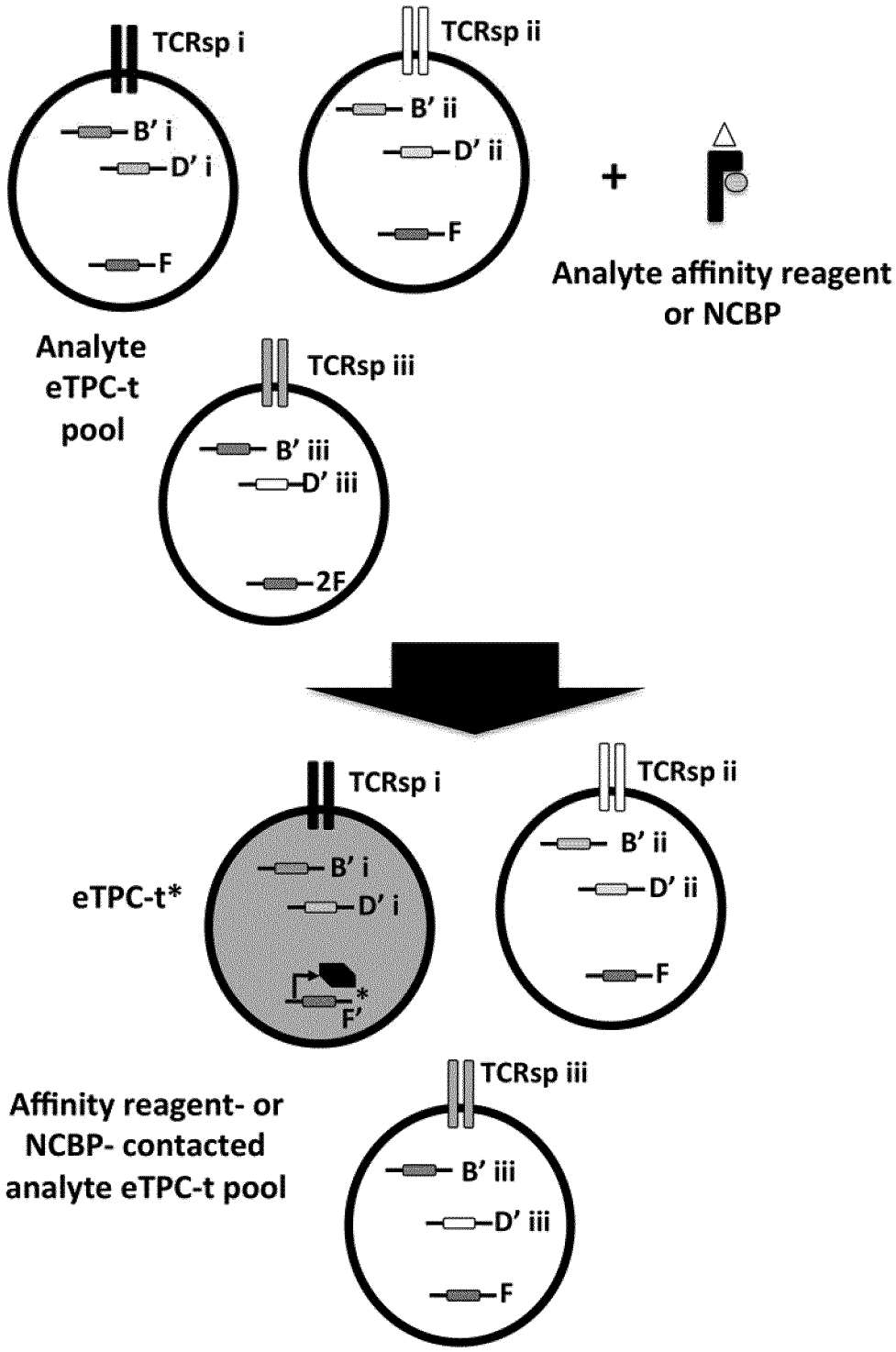

TCRsp i
B' i
D' i
F
TCRsp ii
B' ii
D' ii
F
+
Analyte affinity reagent or NCBP
Analyte eTPC-t pool
TCRsp iii
B' iii
D' iii
2F
eTPC-t*
TCRsp i
B' i
D' i
F'
*
TCRsp ii
B' ii
D' ii
F
Affinity reagent- or NCBP- contacted analyte eTPC-t pool
TCRsp iii
B' iii
D' iii
F

Figure 21

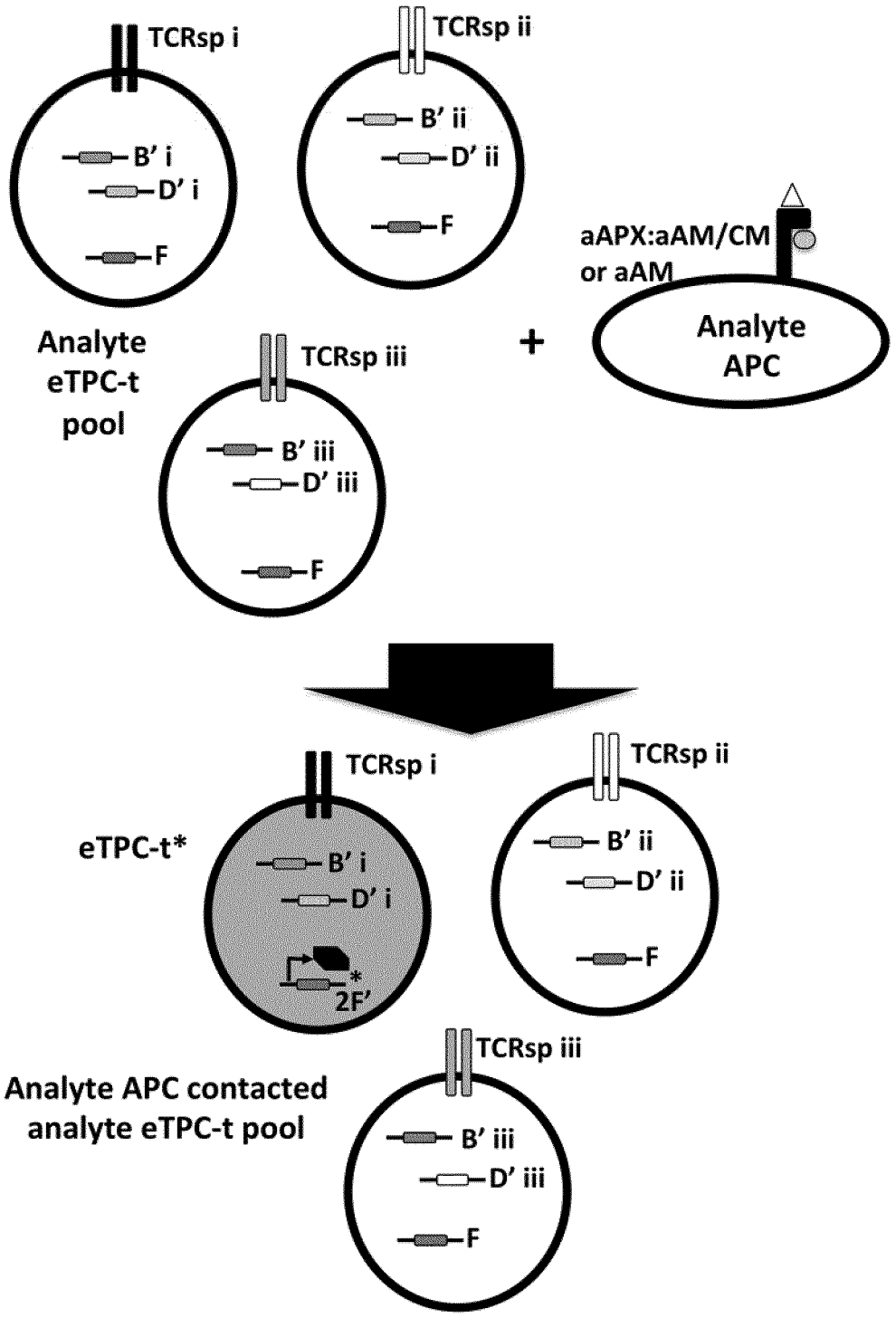

TCRsp i
B' i
D' i
F
TCRsp ii
B' ii
D' ii
F
aAPX:aAM/CM
or aAM
Analyte
APC
+
Analyte
eTPC-t
pool
TCRsp iii
B' iii
D' iii
F
TCRsp i
eTPC-t*
B' i
D' i
*
2F'
TCRsp ii
B' ii
D' ii
F
TCRsp iii
Analyte APC contacted
analyte eTPC-t pool
B' iii
D' iii
F

Figure 22

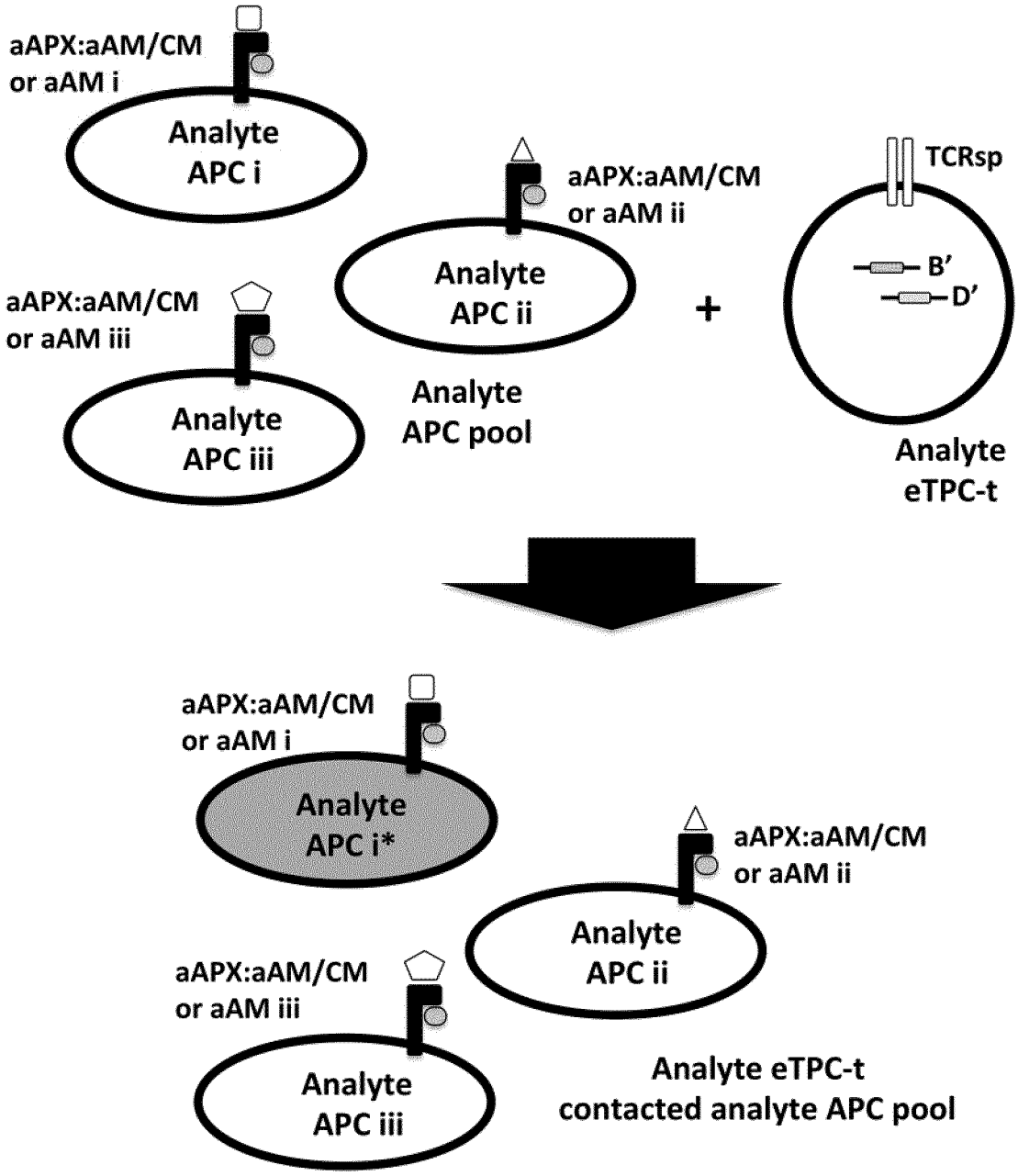

aAPX:aAM/CM
or aAM i
Analyte
APC i
aAPX:aAM/CM
or aAM ii
Analyte
APC ii
TCRsp
B'
D'
aAPX:aAM/CM
or aAM iii
Analyte
APC iii
Analyte
APC pool
+
Analyte
eTPC-t
aAPX:aAM/CM
or aAM i
Analyte
APC i*
aAPX:aAM/CM
or aAM ii
Analyte
APC ii
aAPX:aAM/CM
or aAM iii
Analyte
APC iii
Analyte eTPC-t
contacted analyte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

Figure 23

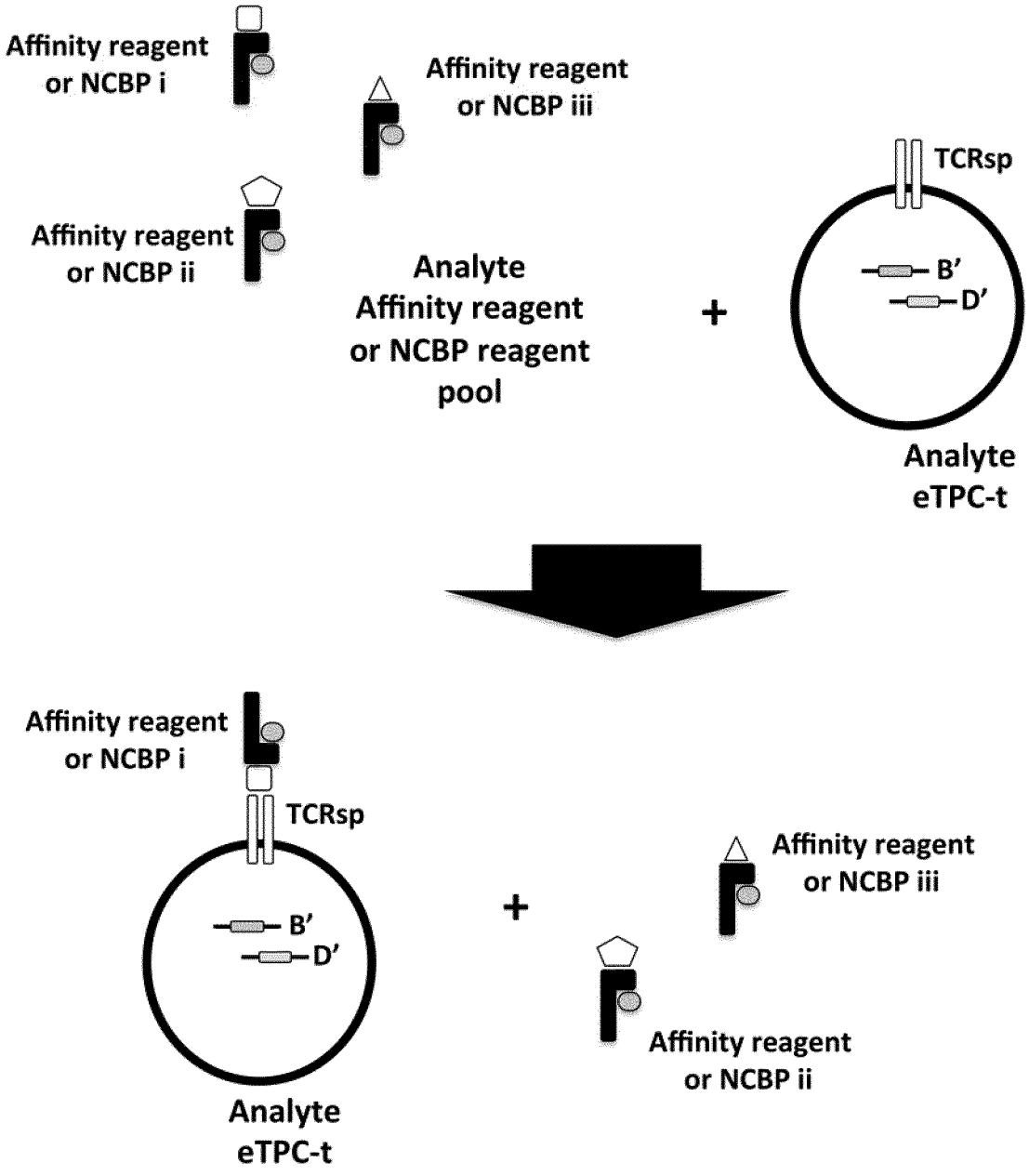

Affinity reagent or NCBP i
Affinity reagent or NCBP iii
Affinity reagent or NCBP ii
Analyte Affinity reagent or NCBP reagent pool
+
TCRsp
B'
D'
Analyte eTPC-t
Affinity reagent or NCBP i
TCRsp
B'
D'
Analyte eTPC-t
+
Affinity reagent or NCBP iii
Affinity reagent or NCBP ii

Figure 24

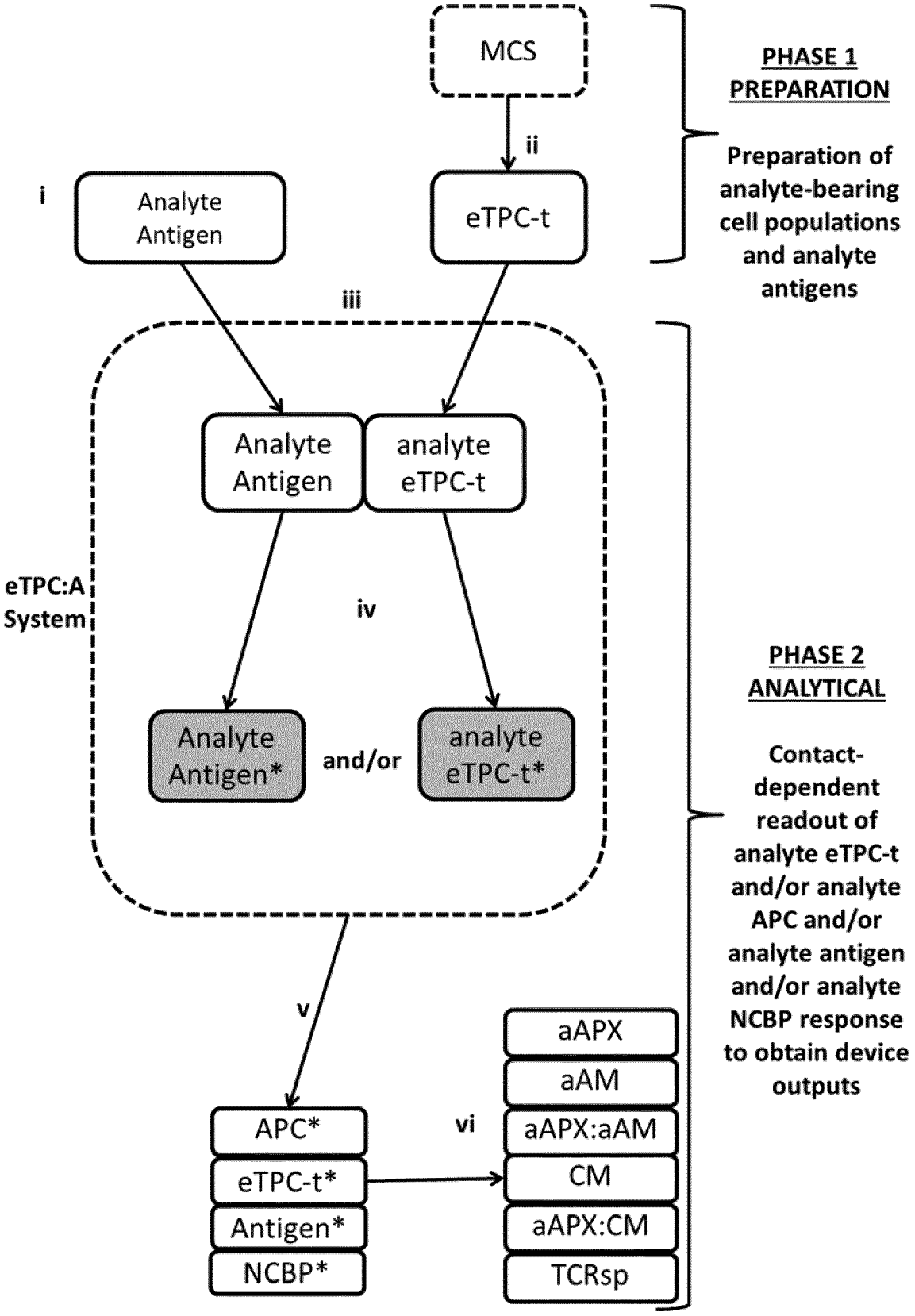

MCS
ii
i
Analyte Antigen
eTPC-t
iii
Analyte Antigen
analyte eTPC-t
eTPC:A System
iv
Analyte Antigen*
and/or
analyte eTPC-t*
v
APC*
eTPC-t*
Antigen*
NCBP*
vi
aAPX
aAM
aAPX:aAM
CM
aAPX:CM
TCRsp
PHASE 1 PREPARATION
Preparation of analyte-bearing cell populations and analyte antigens
PHASE 2 ANALYTICAL
Contact-dependent readout of analyte eTPC-t and/or analyte APC and/or analyte antigen and/or analyte NCBP response to obtain device outputs

Figure 25

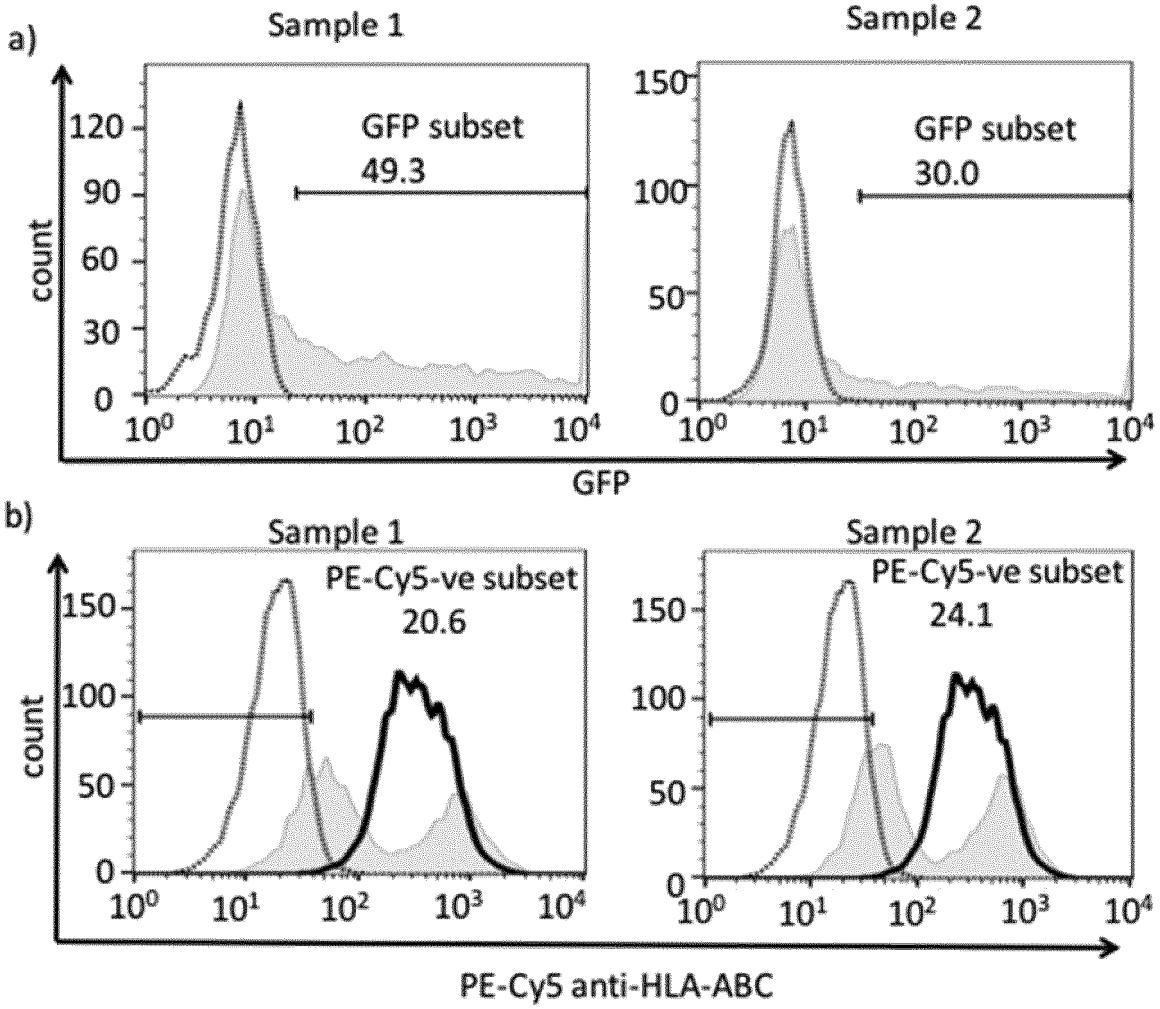

a)
Sample 1
GFP subset
49.3
count
120
90
60
30
0
Sample 2
GFP subset
30.0
150
100
50
0
10^0 10^1 10^2 10^3 10^4
GFP
b)
Sample 1
PE-Cy5-ve subset
20.6
count
150
100
50
0
Sample 2
PE-Cy5-ve subset
24.1
PE-Cy5 anti-HLA-ABC

Figure 26

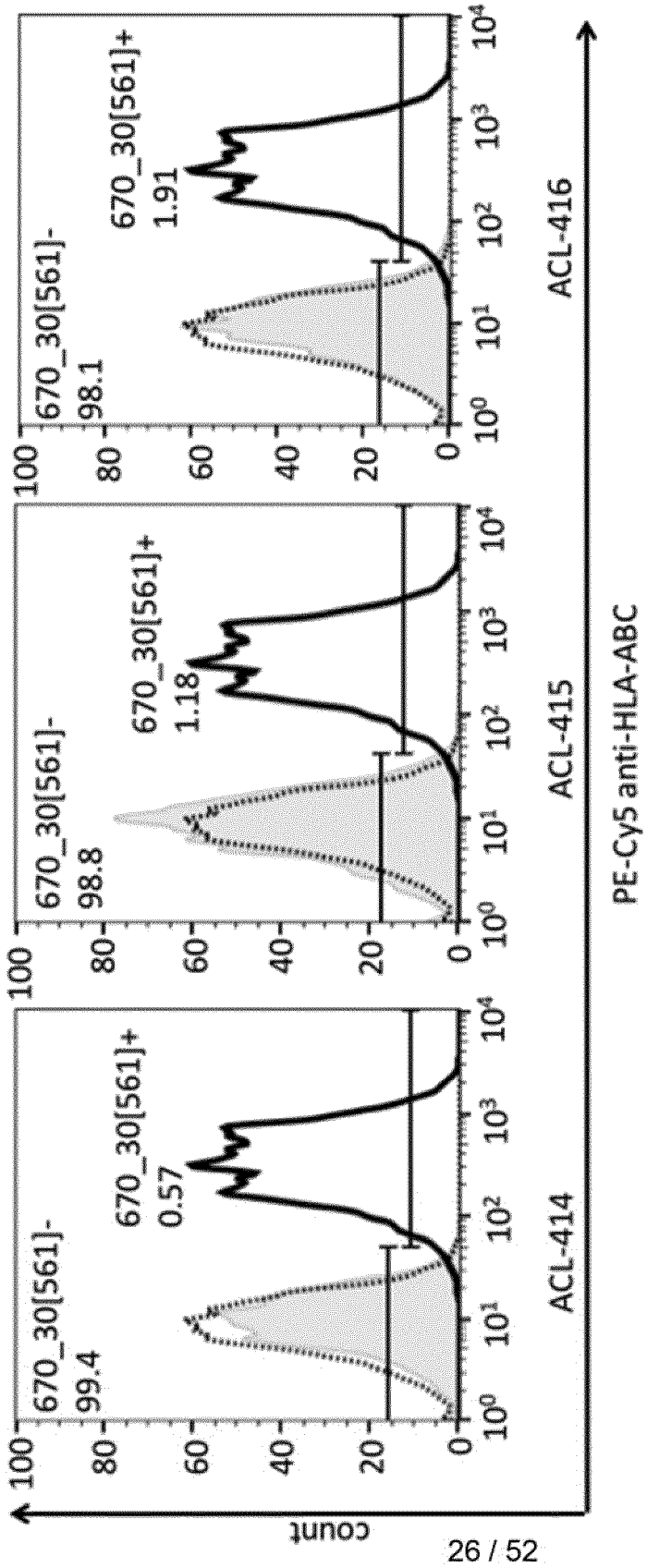

670_30[561]-
99.4
670_30[561]+
0.57
670_30[561]-
98.8
670_30[561]+
1.18
670_30[561]-
98.1
670_30[561]+
1.91
ACL-414
ACL-415
ACL-416
PE-Cy5 anti-HLA-ABC
count

Figure 27

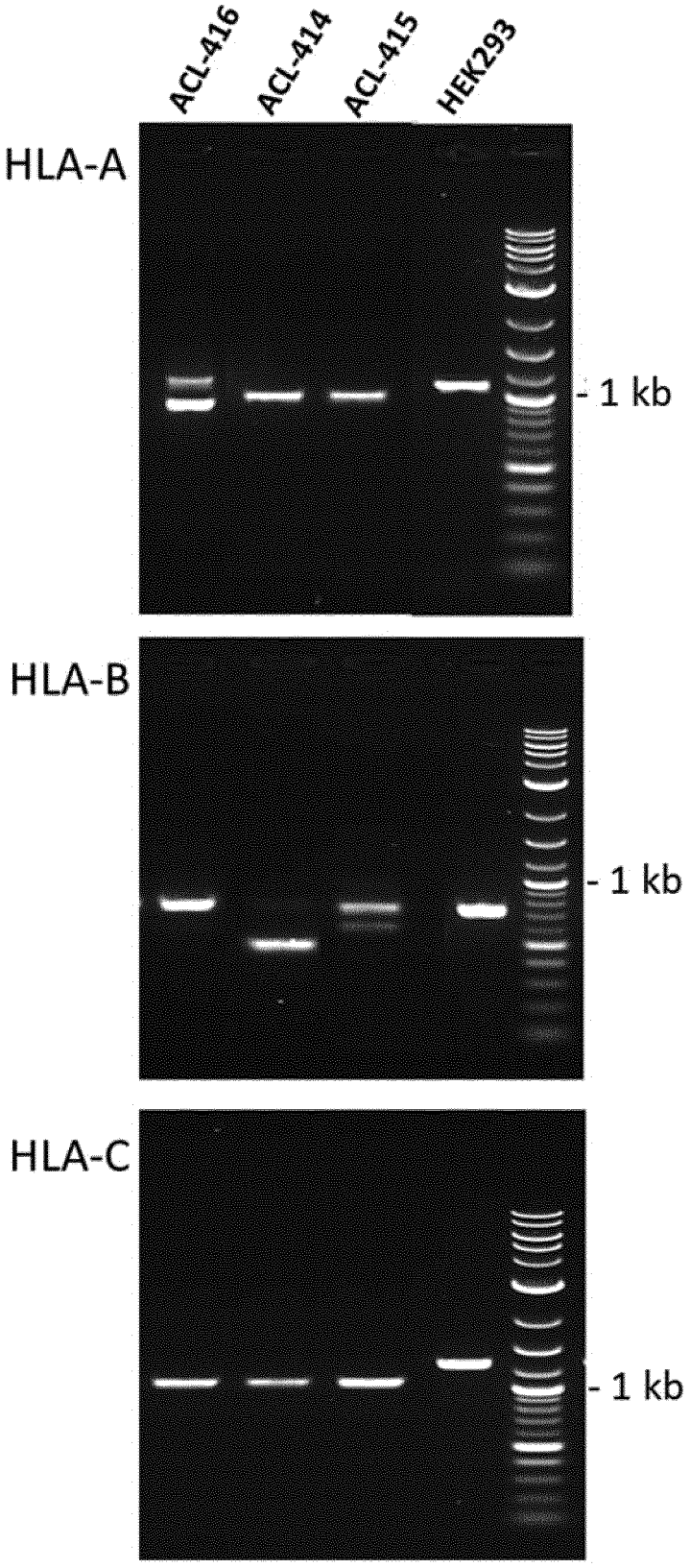


Figure 28

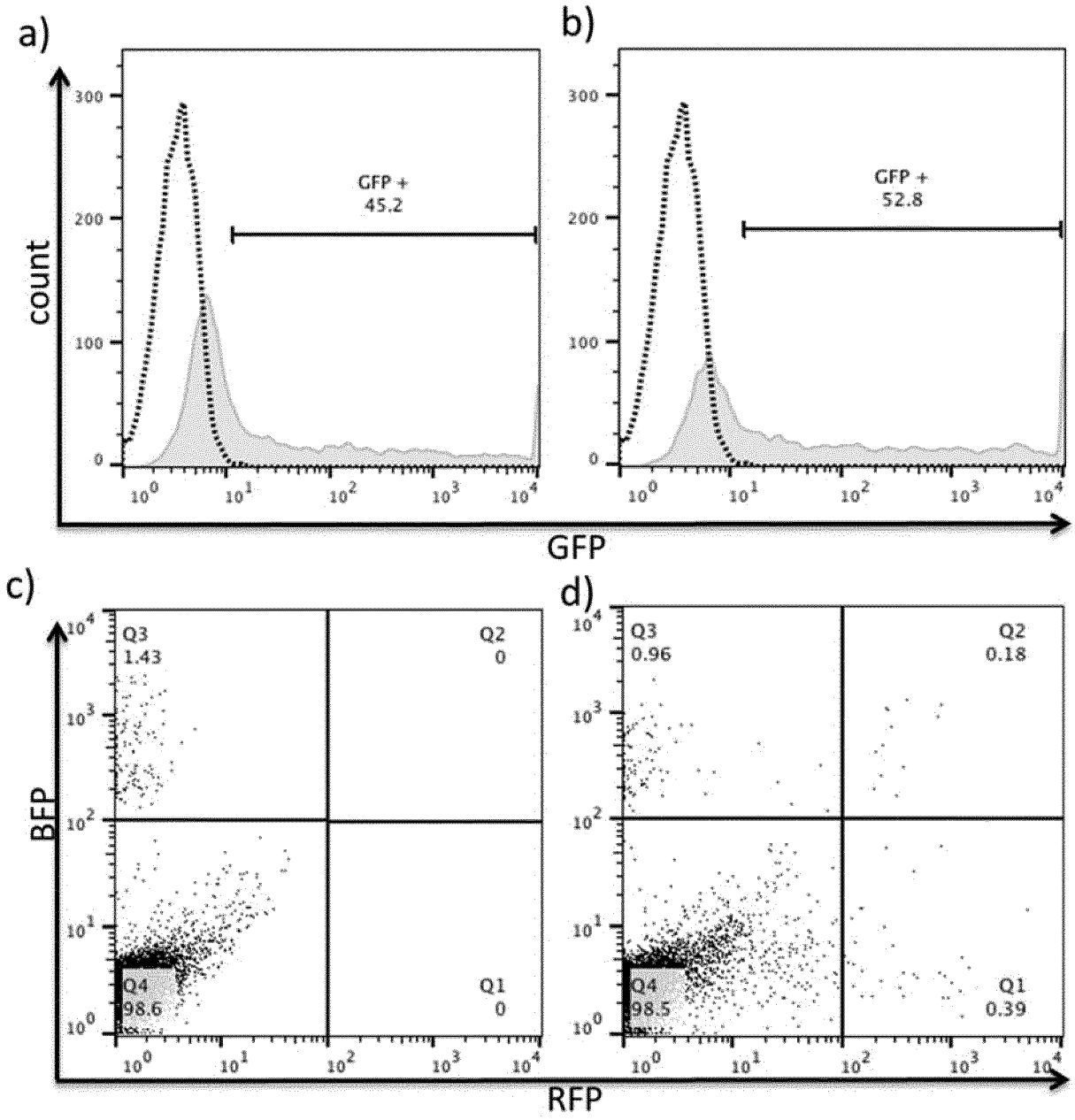

a)
b)
count
GFP +
45.2
GFP +
52.8
GFP
c)
d)
BFP
RFP
Q3
1.43
Q2
0
Q4
98.6
Q1
0
Q3
0.96
Q2
0.18
Q4
98.5
Q1
0.39

Figure 29

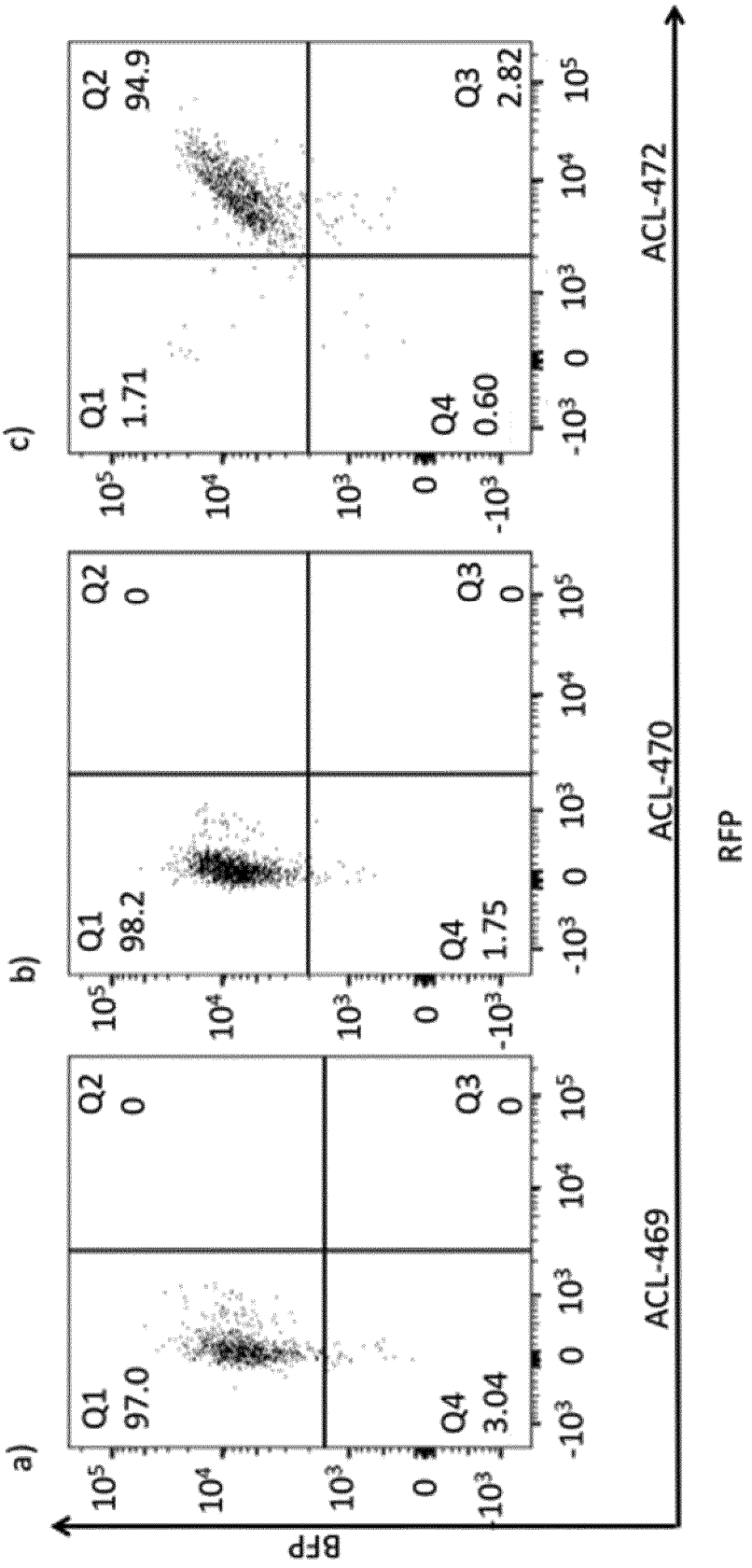

a)
b)
c)
Q1
97.0
Q2
0
Q3
0
Q4
3.04
Q1
98.2
Q2
0
Q3
0
Q4
1.75
Q1
1.71
Q2
94.9
Q3
2.82
Q4
0.60
BFP
ACL-469
ACL-470
ACL-472
RFP

Figure 30

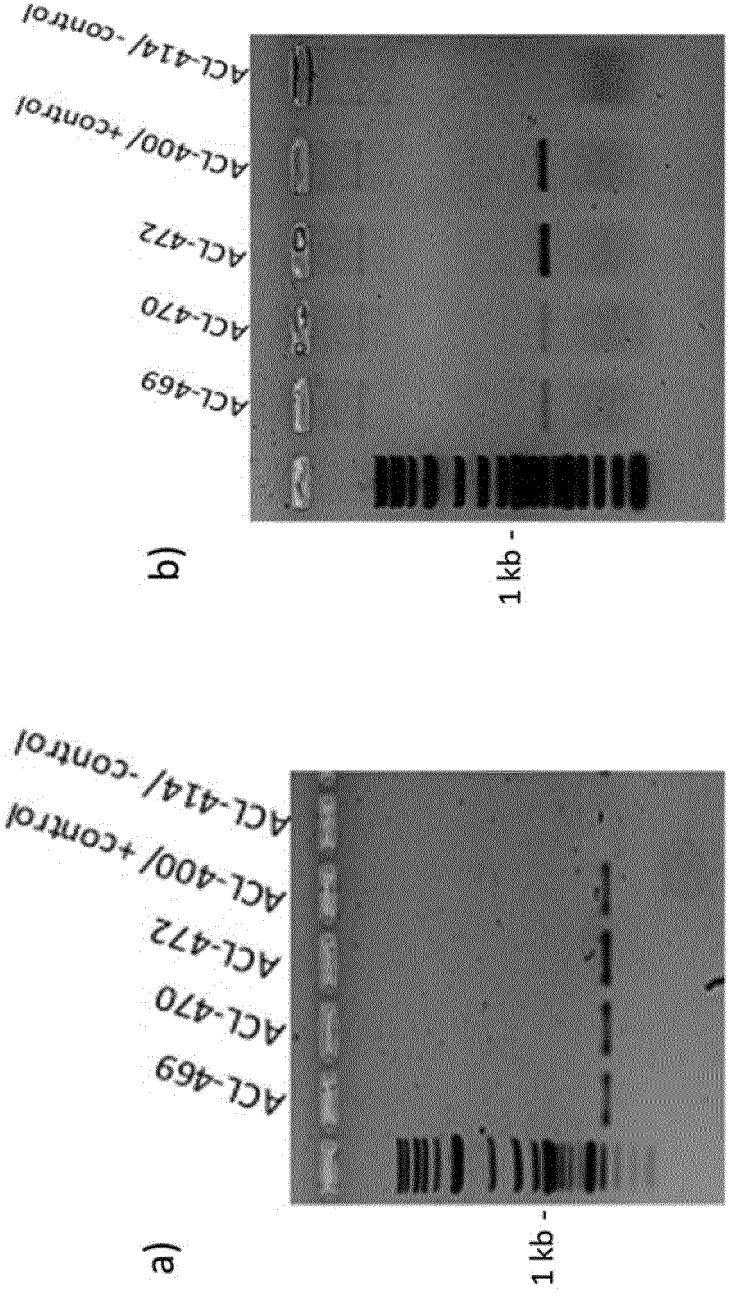

b)
1 kb -
ACL-469
ACL-470
ACL-472
ACL-400/ +control
ACL-414/ -control
a)
1 kb -
ACL-469
ACL-470
ACL-472
ACL-400/ +control
ACL-414/ -control

Figure 31

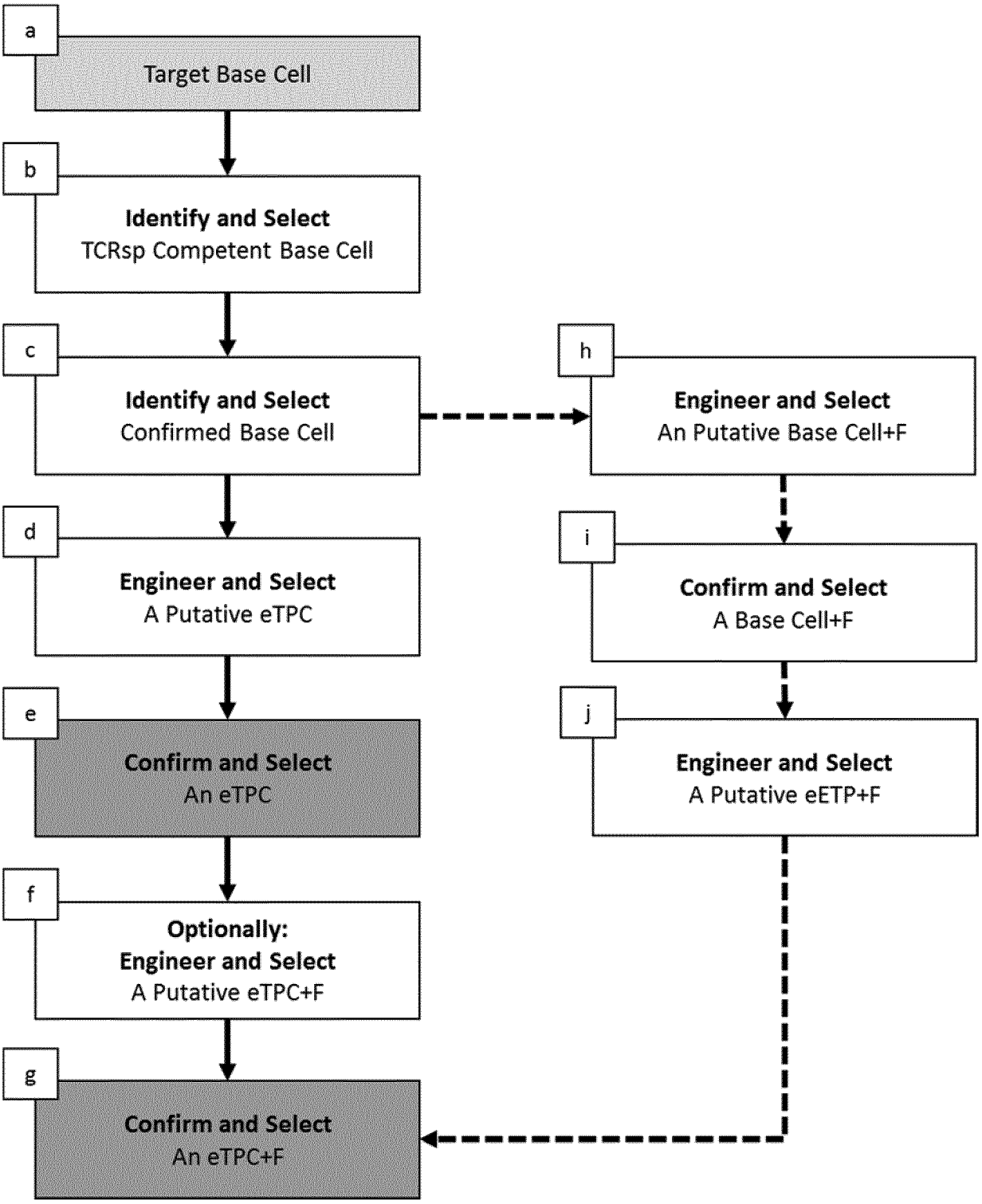

a
Target Base Cell
b
Identify and Select
TCRsp Competent Base Cell
c
Identify and Select
Confirmed Base Cell
d
Engineer and Select
A Putative eTPC
e
Confirm and Select
An eTPC
f
Optionally:
Engineer and Select
A Putative eTPC+F
g
Confirm and Select
An eTPC+F
h
Engineer and Select
An Putative Base Cell+F
i
Confirm and Select
A Base Cell+F
j
Engineer and Select
A Putative eETP+F

Figure 32

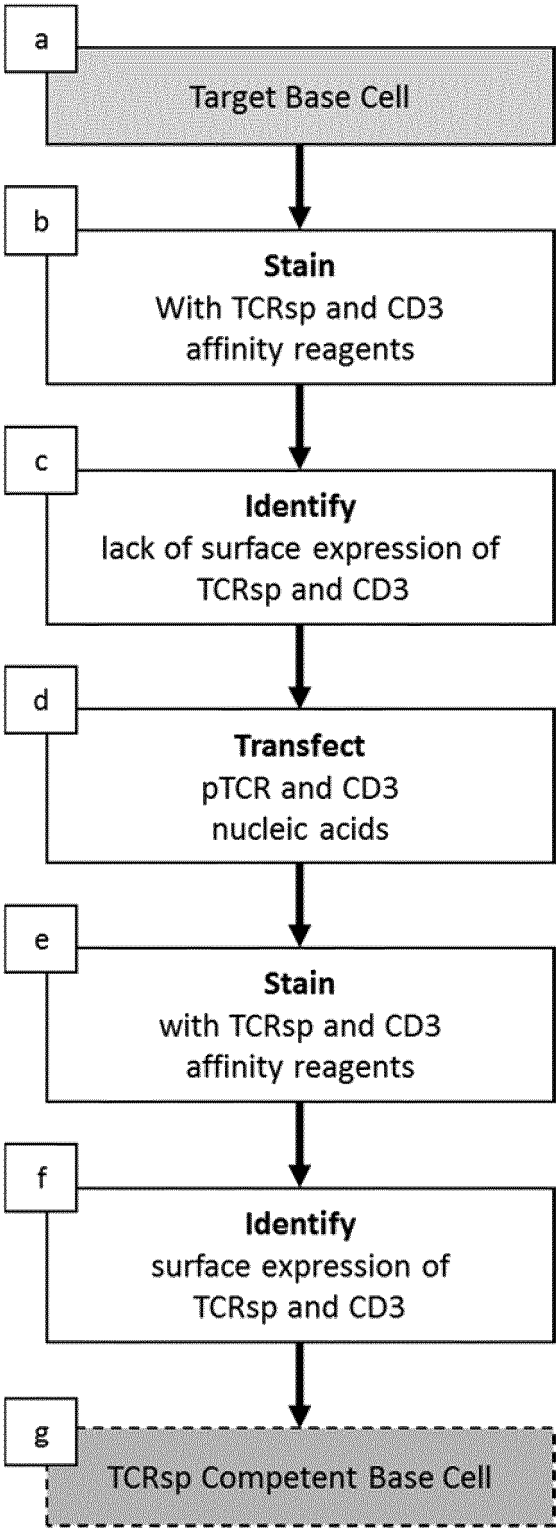

a
Target Base Cell
b
Stain
With TCRsp and CD3
affinity reagents
c
Identify
lack of surface expression of
TCRsp and CD3
d
Transfect
pTCR and CD3
nucleic acids
e
Stain
with TCRsp and CD3
affinity reagents
f
Identify
surface expression of
TCRsp and CD3
g
TCRsp Competent Base Cell

Figure 33

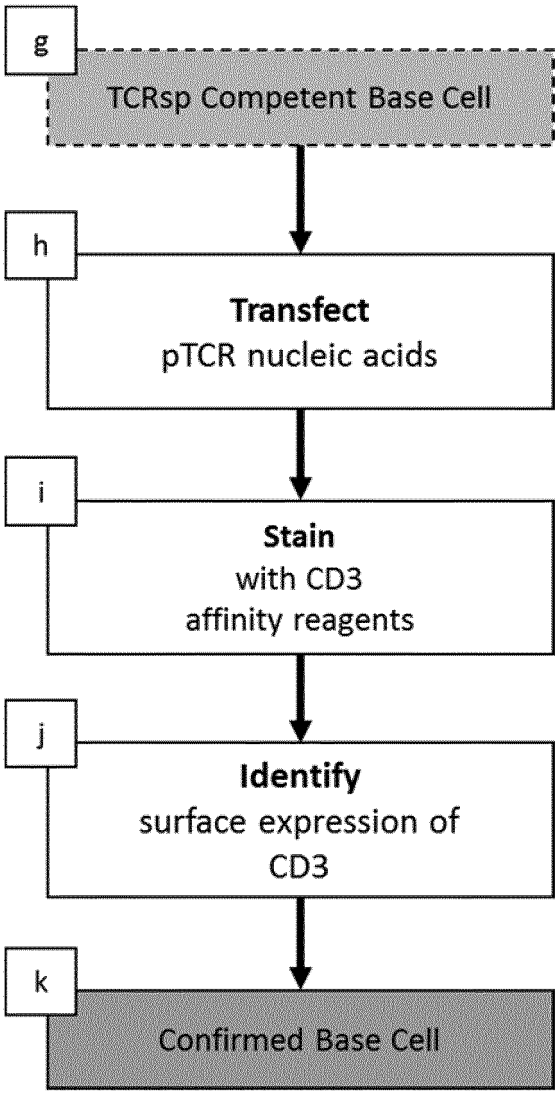

g
TCRsp Competent Base Cell
h
Transfect
pTCR nucleic acids
i
Stain
with CD3
affinity reagents
j
Identify
surface expression of
CD3
k
Confirmed Base Cell

Figure 34

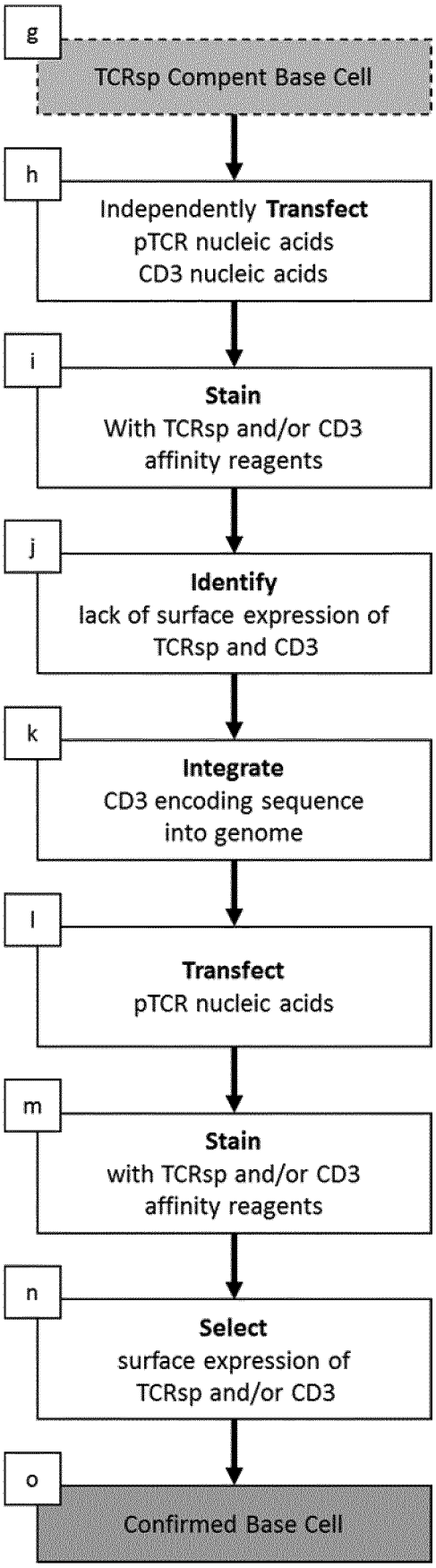

g
TCRsp Compent Base Cell
h
Independently **Transfect**
pTCR nucleic acids
CD3 nucleic acids
i
Stain
With TCRsp and/or CD3
affinity reagents
j
Identify
lack of surface expression of
TCRsp and CD3
k
Integrate
CD3 encoding sequence
into genome
l
Transfect
pTCR nucleic acids
m
Stain
with TCRsp and/or CD3
affinity reagents
n
Select
surface expression of
TCRsp and/or CD3
o
Confirmed Base Cell

Figure 35

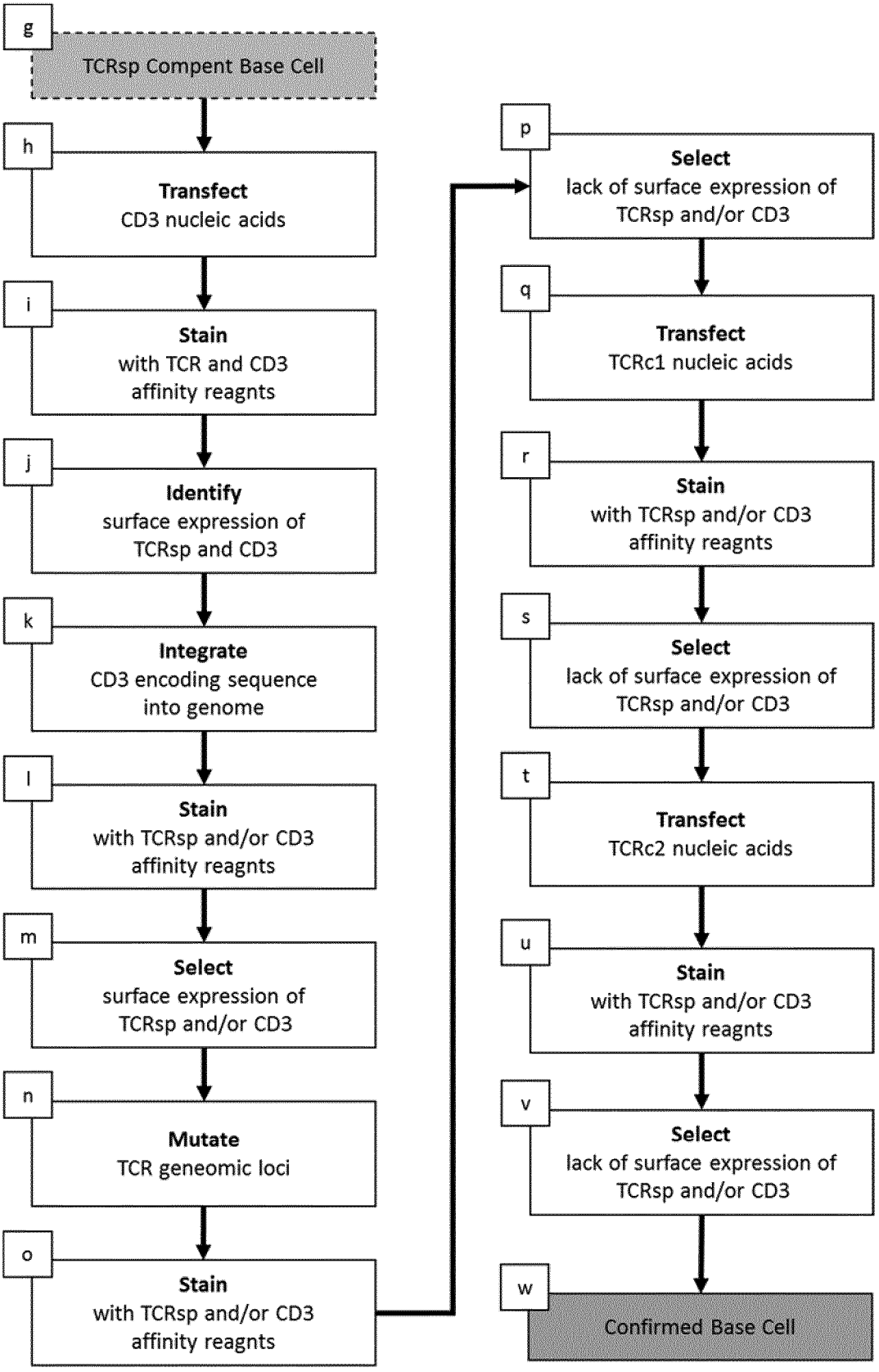

g
TCRsp Compent Base Cell
h
Transfect
CD3 nucleic acids
i
Stain
with TCR and CD3
affinity reagnts
j
Identify
surface expression of
TCRsp and CD3
k
Integrate
CD3 encoding sequence
into genome
l
Stain
with TCRsp and/or CD3
affinity reagnts
m
Select
surface expression of
TCRsp and/or CD3
n
Mutate
TCR geneomic loci
o
Stain
with TCRsp and/or CD3
affinity reagnts
p
Select
lack of surface expression of
TCRsp and/or CD3
q
Transfect
TCRc1 nucleic acids
r
Stain
with TCRsp and/or CD3
affinity reagnts
s
Select
lack of surface expression of
TCRsp and/or CD3
t
Transfect
TCRc2 nucleic acids
u
Stain
with TCRsp and/or CD3
affinity reagnts
v
Select
lack of surface expression of
TCRsp and/or CD3
w
Confirmed Base Cell

Figure 36

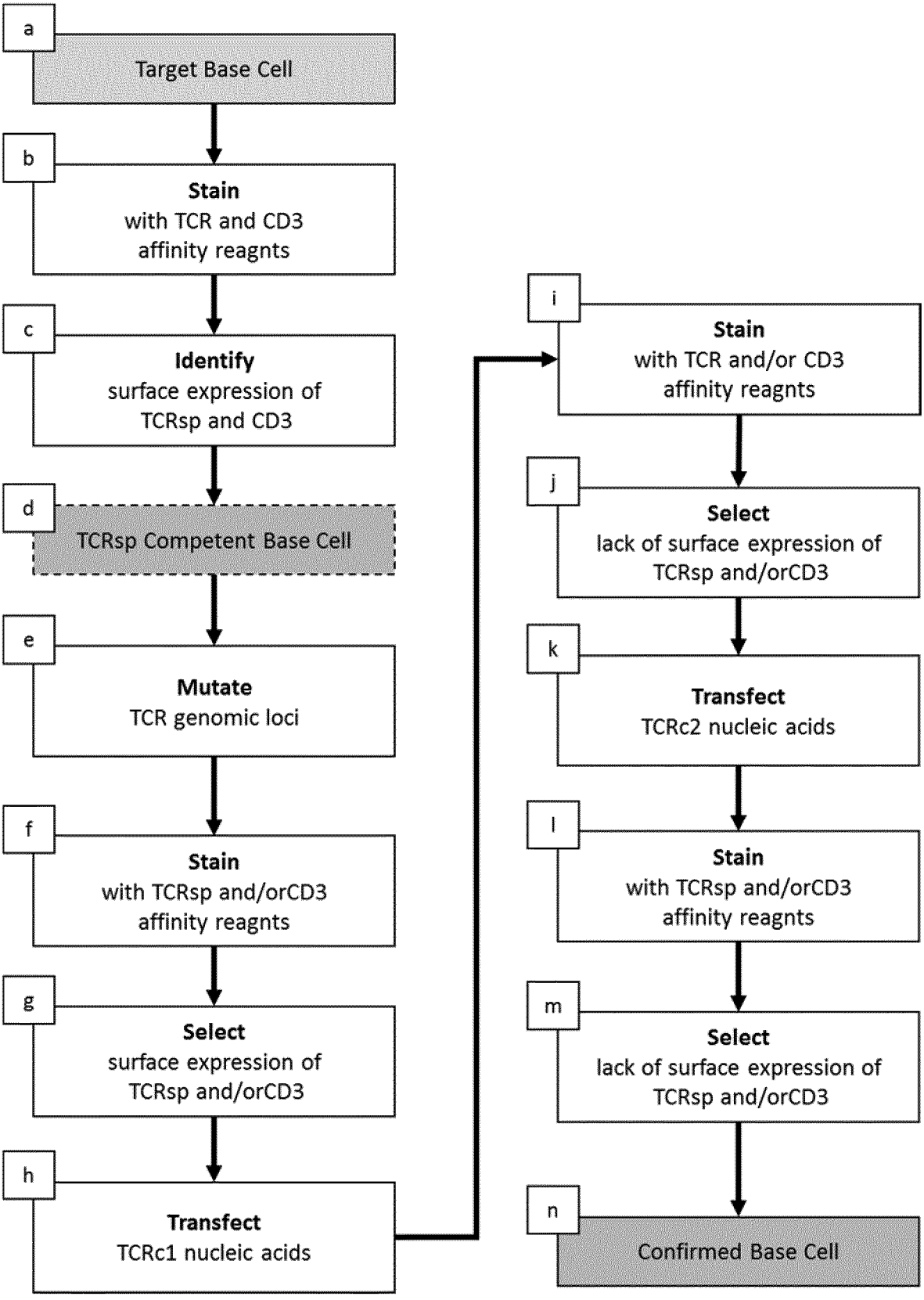

a
Target Base Cell
b
Stain
with TCR and CD3
affinity reagnts
c
Identify
surface expression of
TCRsp and CD3
d
TCRsp Competent Base Cell
e
Mutate
TCR genomic loci
f
Stain
with TCRsp and/orCD3
affinity reagnts
g
Select
surface expression of
TCRsp and/orCD3
h
Transfect
TCRc1 nucleic acids
i
Stain
with TCR and/or CD3
affinity reagnts
j
Select
lack of surface expression of
TCRsp and/orCD3
k
Transfect
TCRc2 nucleic acids
l
Stain
with TCRsp and/orCD3
affinity reagnts
m
Select
lack of surface expression of
TCRsp and/orCD3
n
Confirmed Base Cell

Figure 37

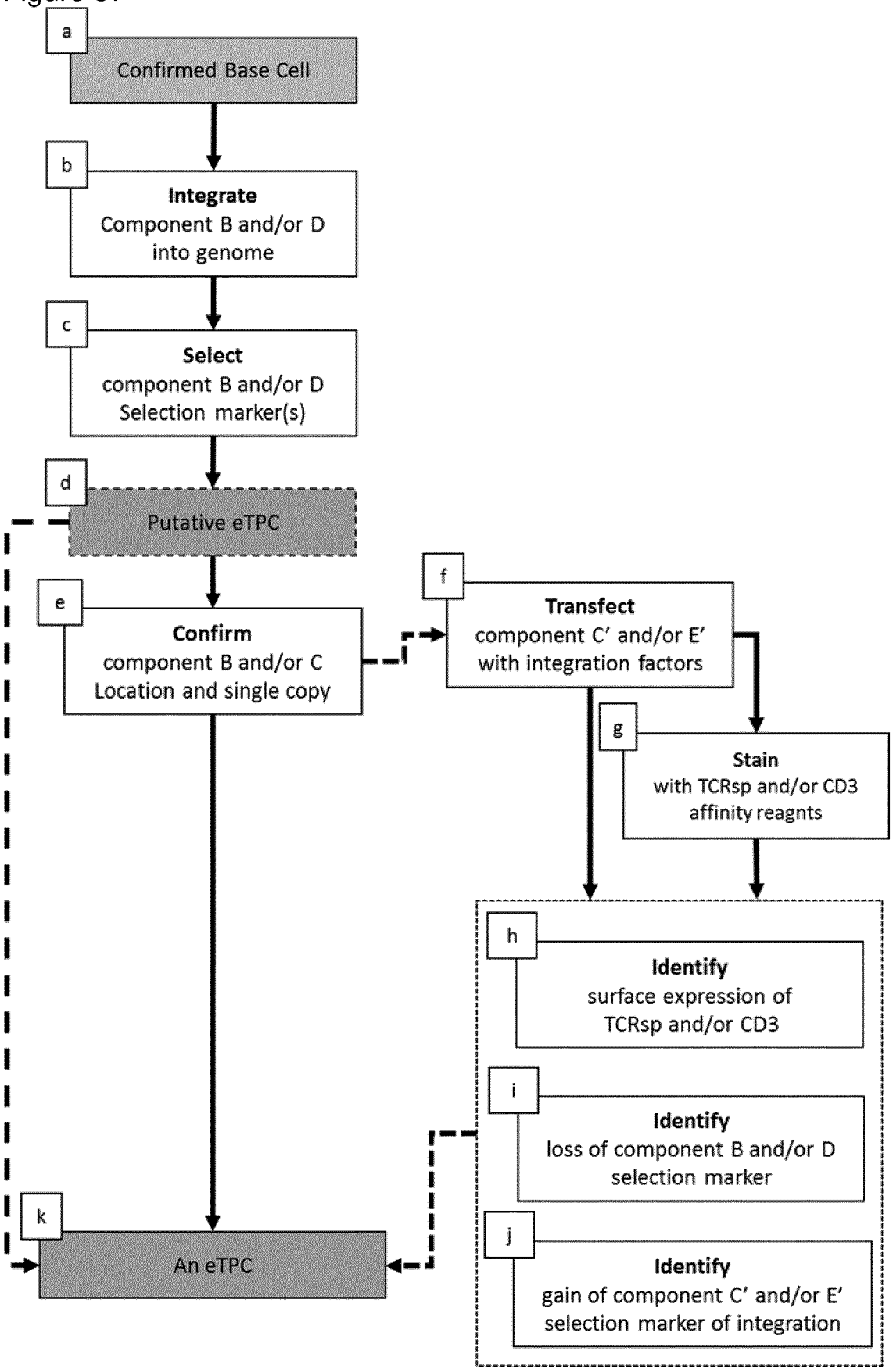

a
Confirmed Base Cell
b
Integrate
Component B and/or D
into genome
c
Select
component B and/or D
Selection marker(s)
d
Putative eTPC
e
Confirm
component B and/or C
Location and single copy
f
Transfect
component C' and/or E'
with integration factors
g
Stain
with TCRsp and/or CD3
affinity reagnts
h
Identify
surface expression of
TCRsp and/or CD3
i
Identify
loss of component B and/or D
selection marker
j
Identify
gain of component C' and/or E'
selection marker of integration
k
An eTPC

Figure 38

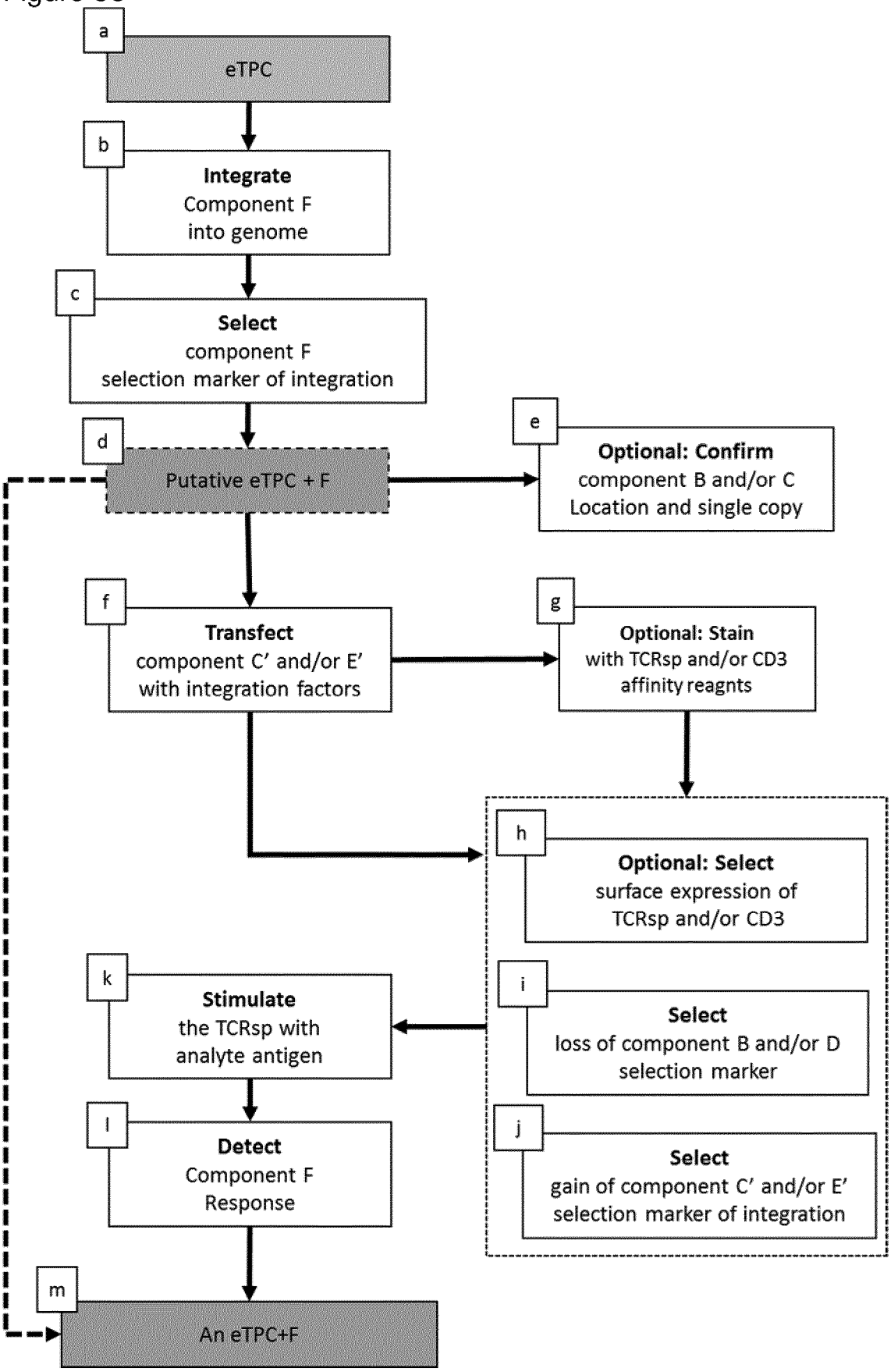

a
eTPC
b
Integrate
Component F
into genome
c
Select
component F
selection marker of integration
d
Putative eTPC + F
e
Optional: Confirm
component B and/or C
Location and single copy
f
Transfect
component C' and/or E'
with integration factors
g
Optional: Stain
with TCRsp and/or CD3
affinity reagnts
h
Optional: Select
surface expression of
TCRsp and/or CD3
i
Select
loss of component B and/or D
selection marker
j
Select
gain of component C' and/or E'
selection marker of integration
k
Stimulate
the TCRsp with
analyte antigen
l
Detect
Component F
Response
m
An eTPC+F

Figure 39

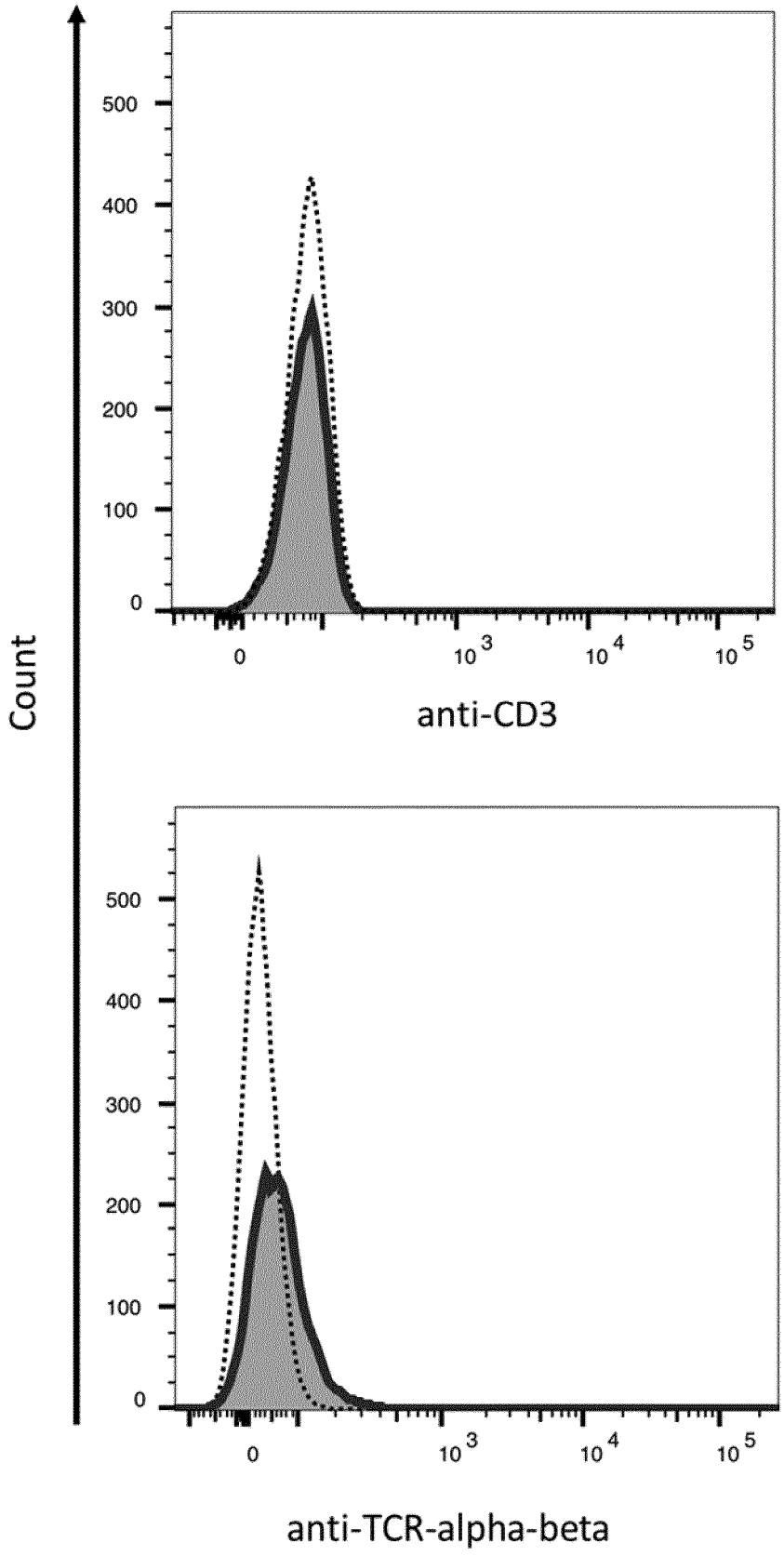

Count
500
400
300
200
100
0
0
10 3
10 4
10 5
anti-CD3
500
400
300
200
100
0
0
10 3
10 4
10 5
anti-TCR-alpha-beta

Figure 40

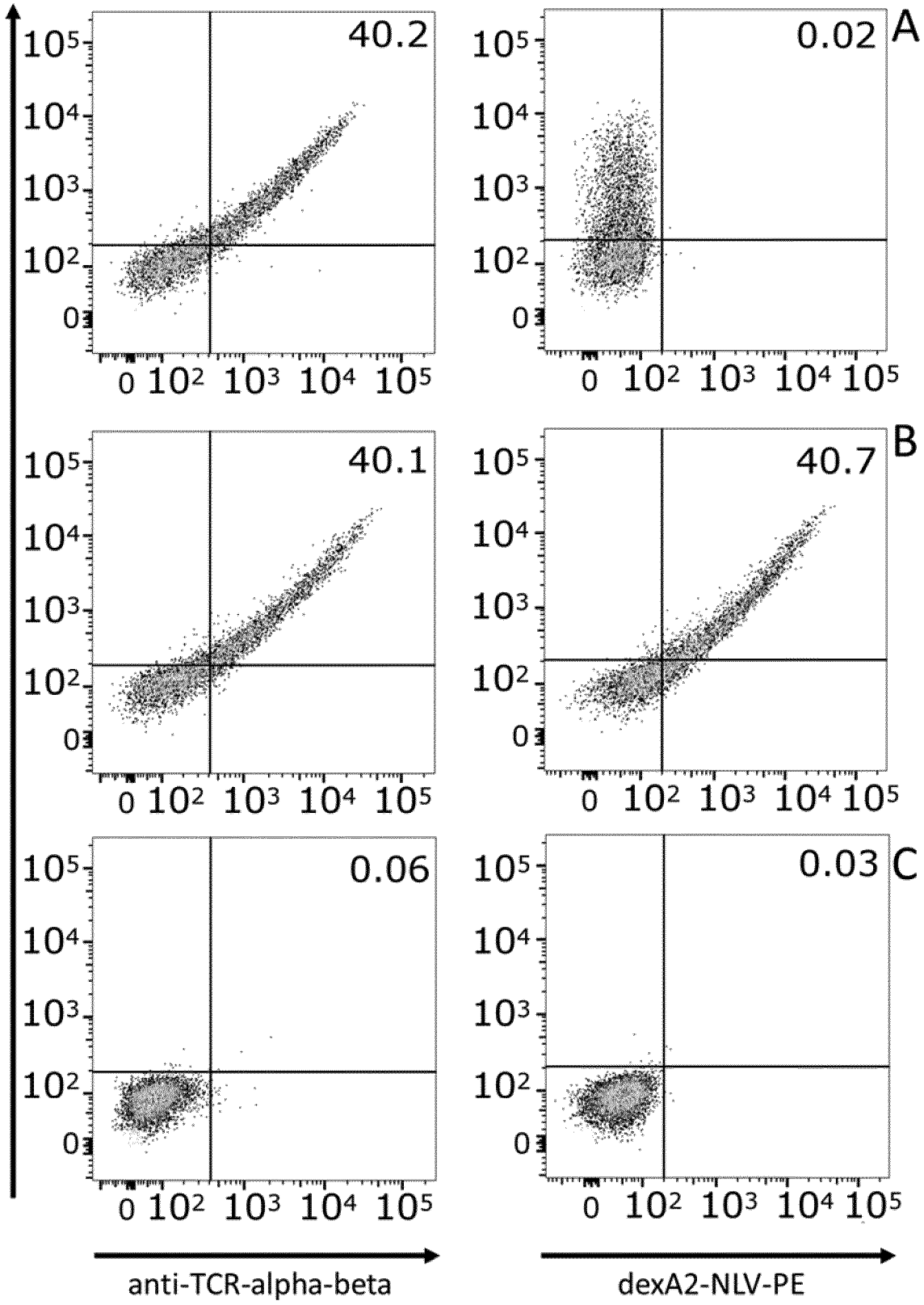

40.2
0.02
A
40.1
40.7
B
0.06
0.03
C
10^5
10^4
10^3
10^2
0
0 10^2 10^3 10^4 10^5
anti-TCR-alpha-beta
dexA2-NLV-PE

Figure 41

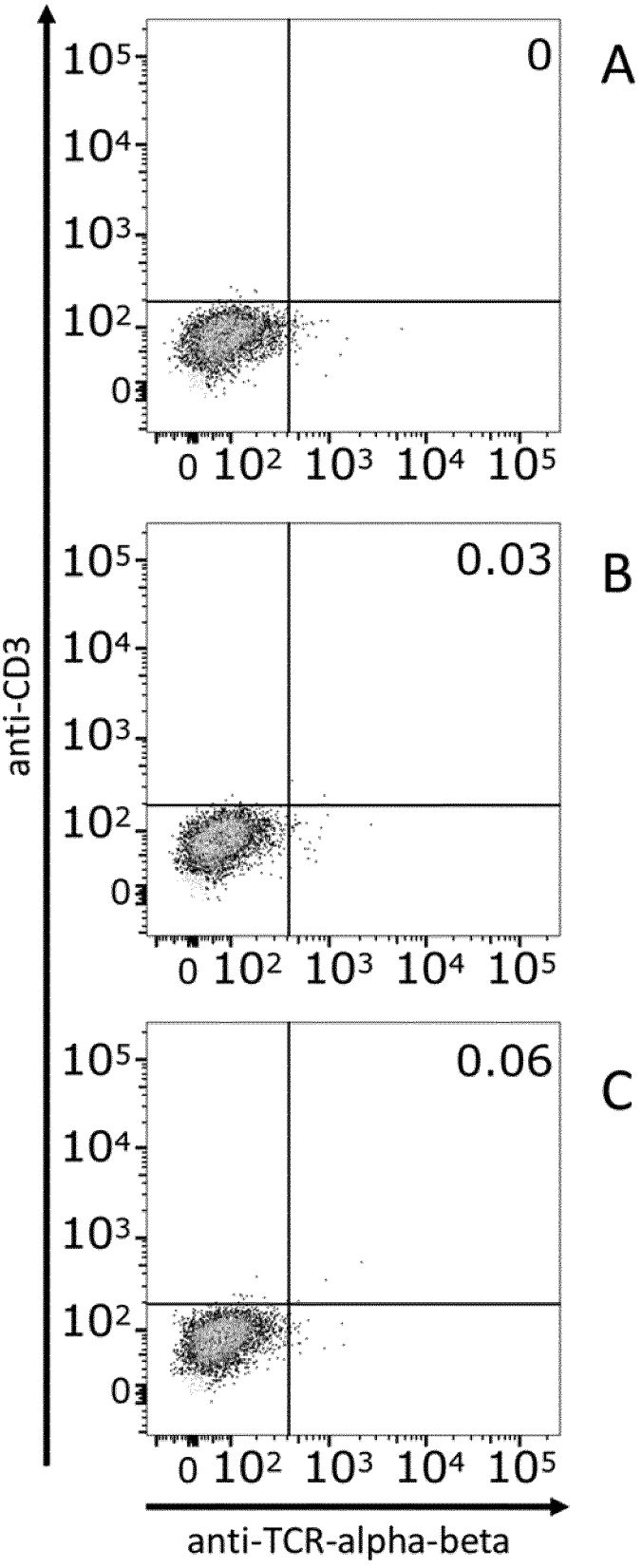


Figure 42

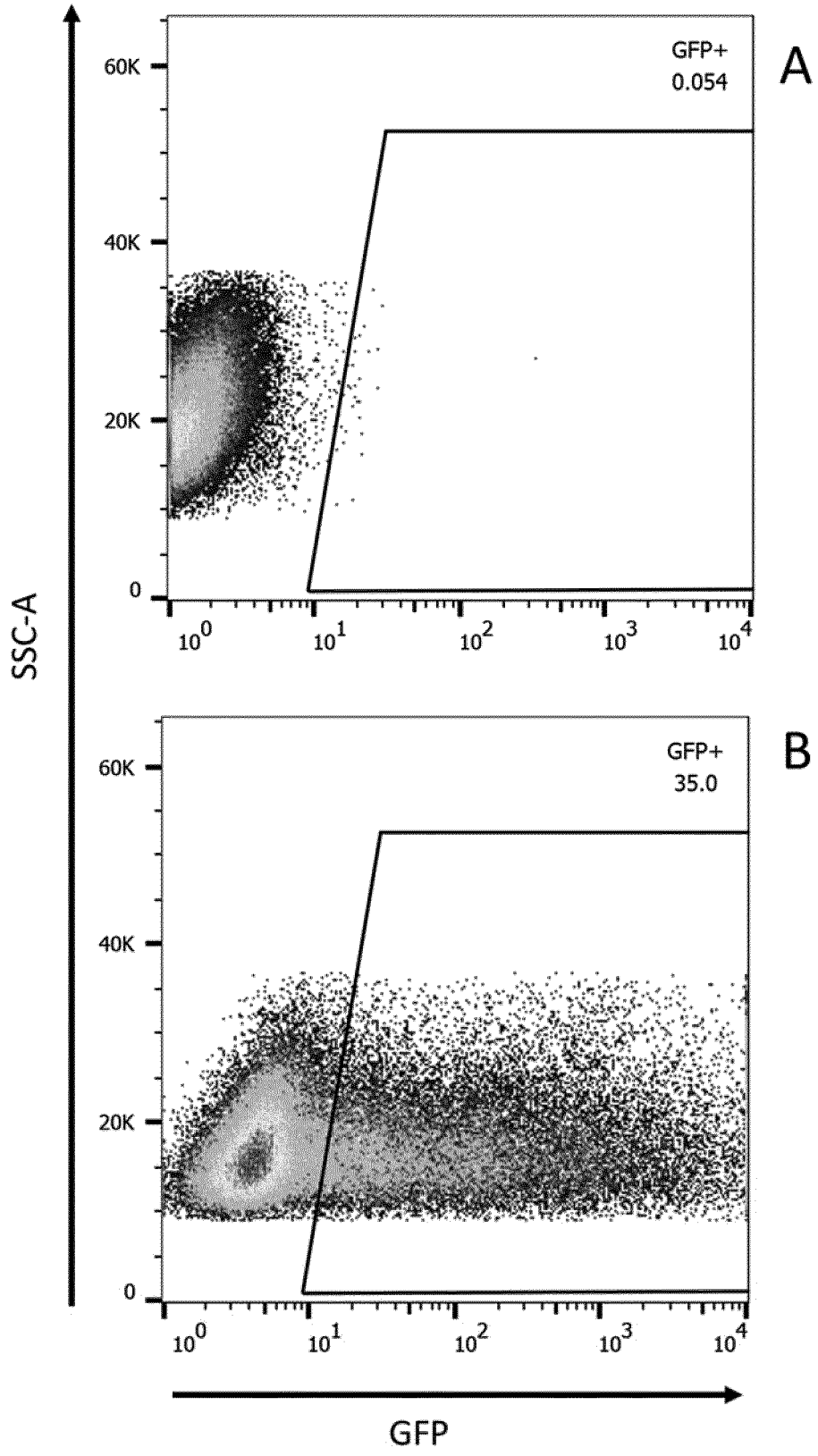

GFP+
0.054
A
60K
40K
20K
0
GFP+
35.0
B
60K
40K
20K
0
10^0
10^1
10^2
10^3
10^4
SSC-A
GFP

Figure 43

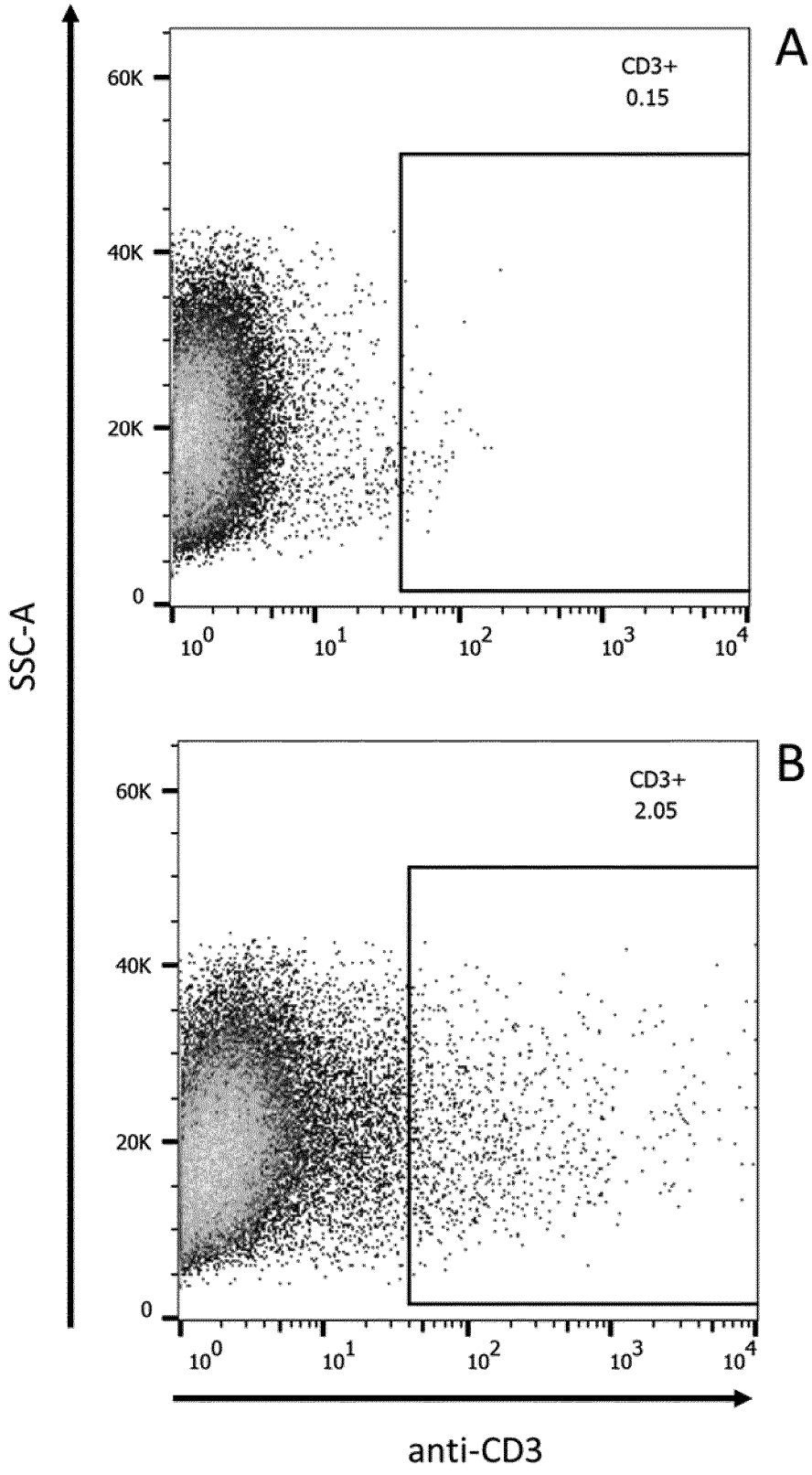

A
CD3+
0.15
60K
40K
20K
0
B
CD3+
2.05
60K
40K
20K
0
10^0
10^1
10^2
10^3
10^4
SSC-A
anti-CD3

Figure 44

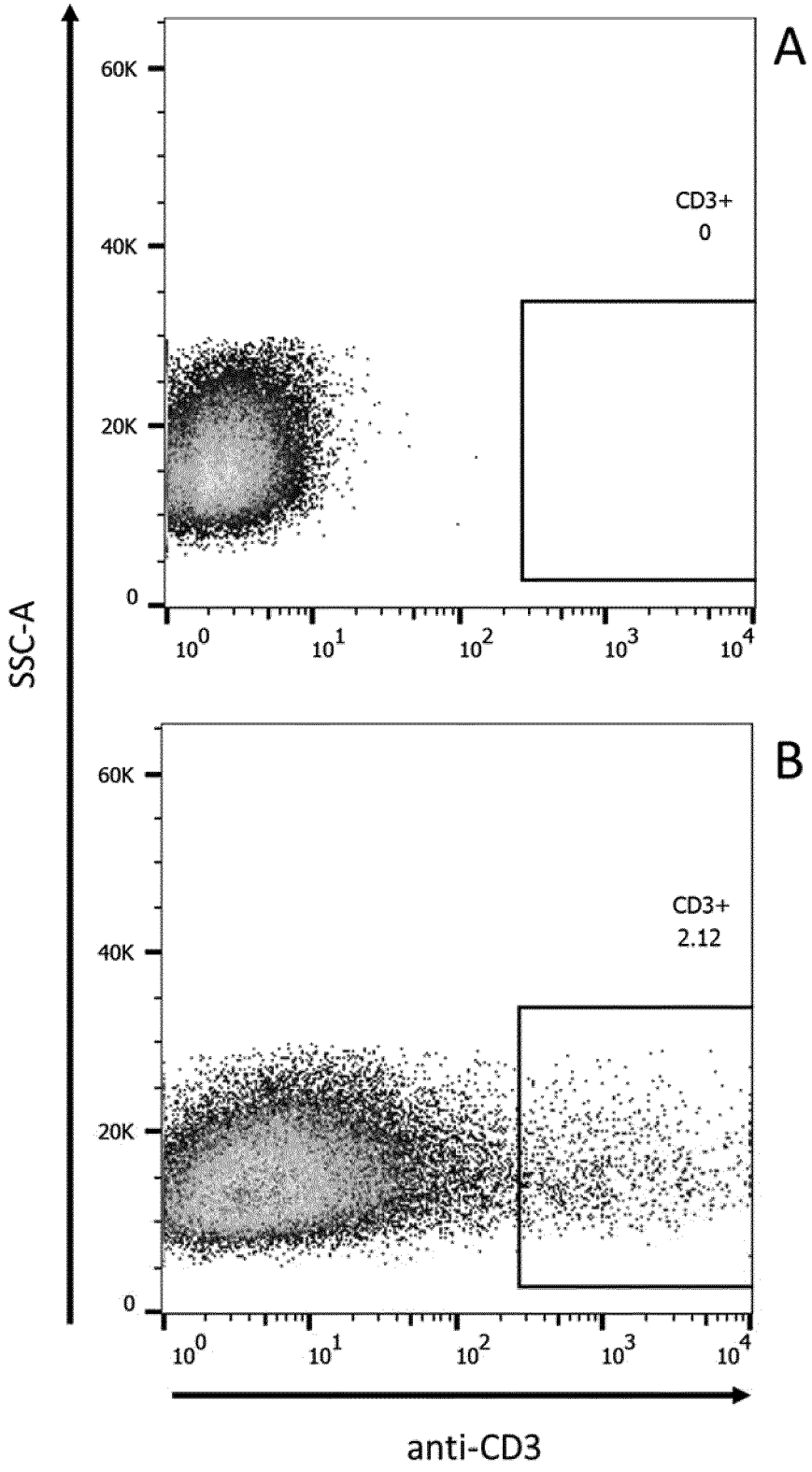

A
60K
40K
20K
0
CD3+
0
B
60K
40K
20K
0
CD3+
2.12
SSC-A
10^0
10^1
10^2
10^3
10^4
anti-CD3

Figure 45

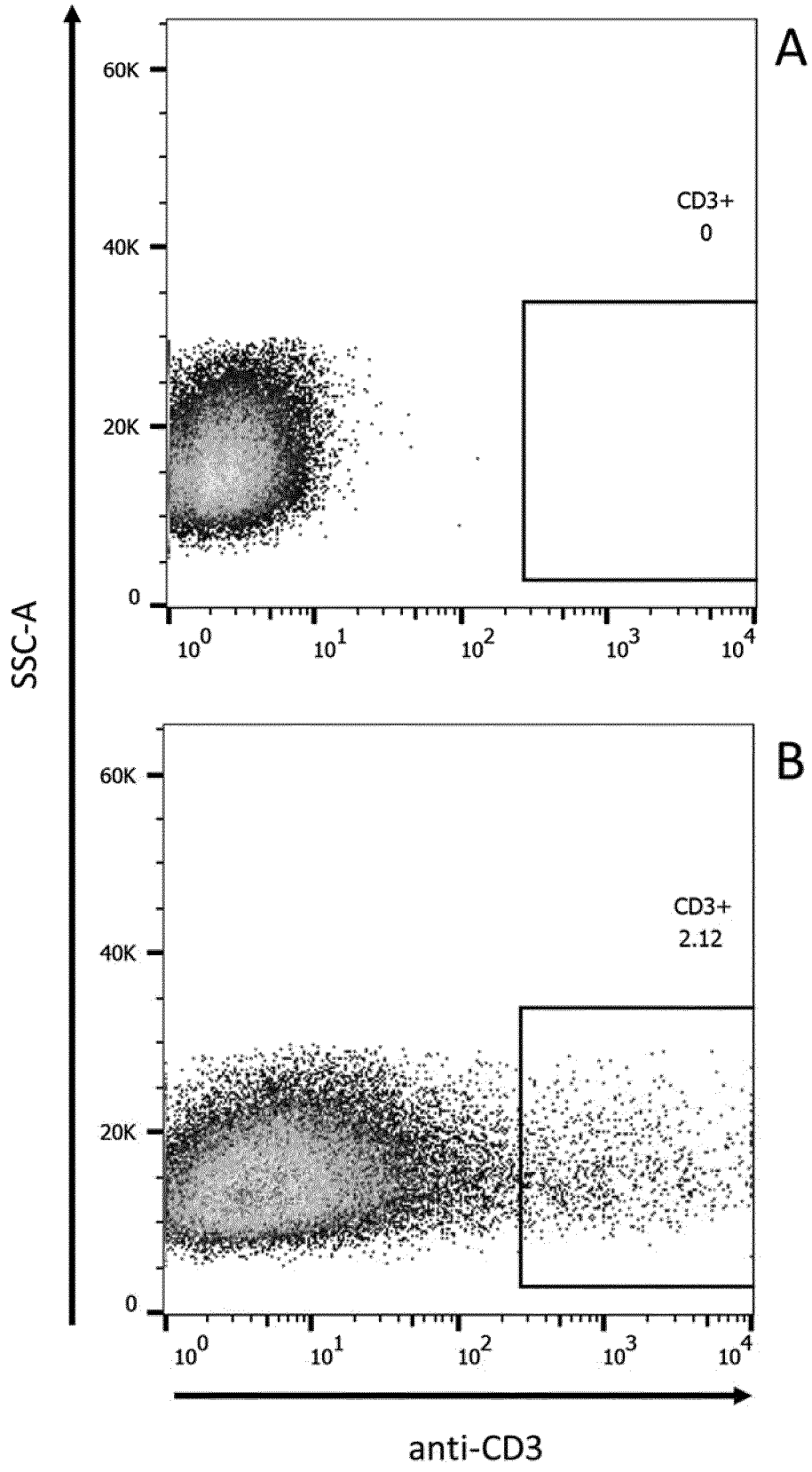

A
60K
40K
20K
0
CD3+
0
B
60K
40K
20K
0
CD3+
2.12
SSC-A
10^0
10^1
10^2
10^3
10^4
anti-CD3

Figure 46

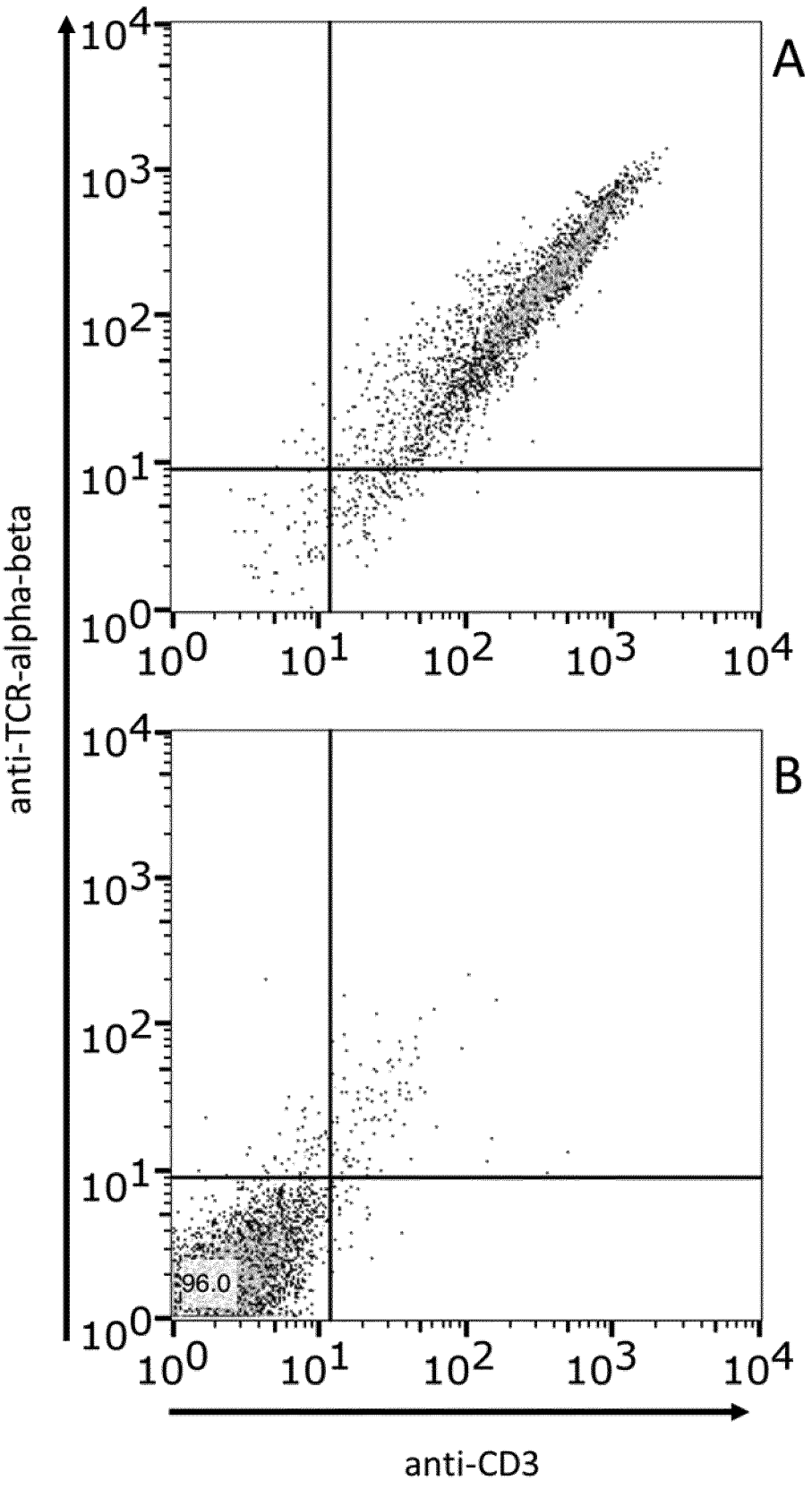


Figure 47

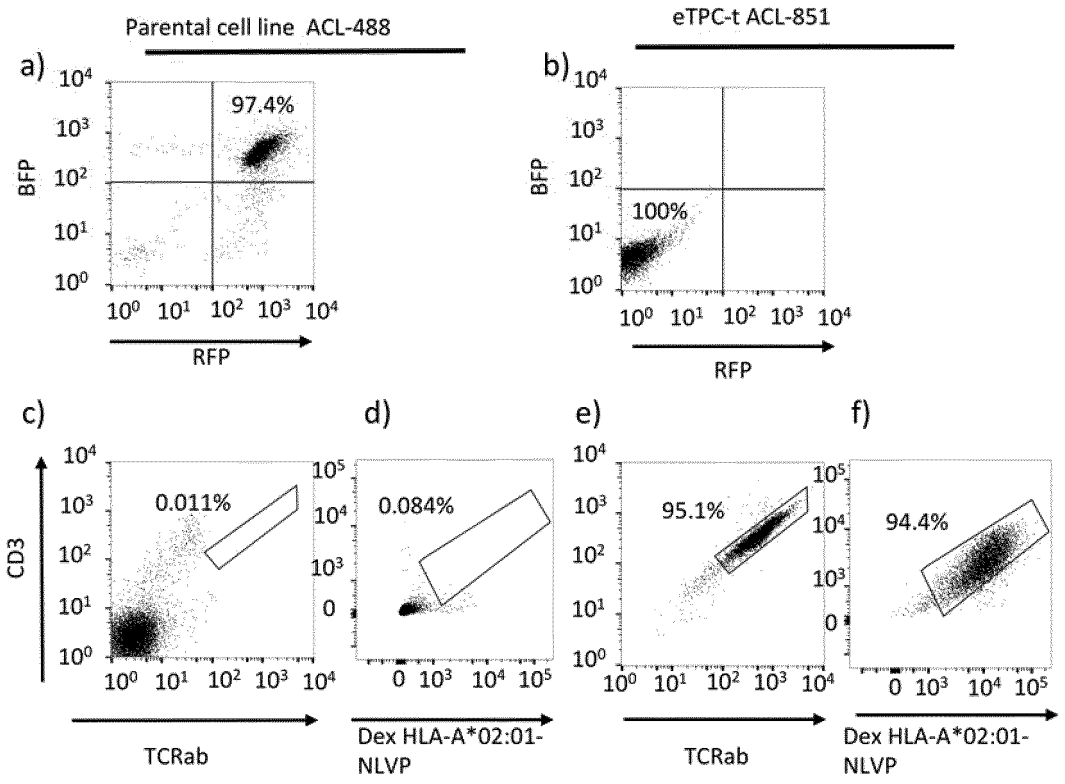
Table 1. Genetic characterization of monoclones containing component B

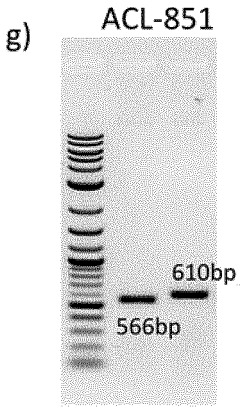
Cell Line	Genomic location		Copy number of integrated component B			
	Integrated into the genome	Integrated into the genomic integration site (AAVS1)	Reference gene copies/ul	Component B copies/ul	Observed Ratio	Expected ratio for a single integration of component B
ACL-469	Yes	Yes	151	51.8	**0,343**	**0,33**
ACL-470	Yes	Yes	166	56.8	**0,342**	**0,33**

Table 2. Genetic characterization of monoclones containing components B and D

Cell Line	Genomic location		Copy number of integrated component B and D			
	Integrated into the genome	Integrated into the genomic integration site (AAVS1)	Reference gene copies/ul	Component B and D copies/ul	Observed Ratio	Expected ratio for a single integration of component B and D
ACL-472	Yes	**Yes**	**183**	**124**	**0,675**	**0,66**

Figure 48





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 49

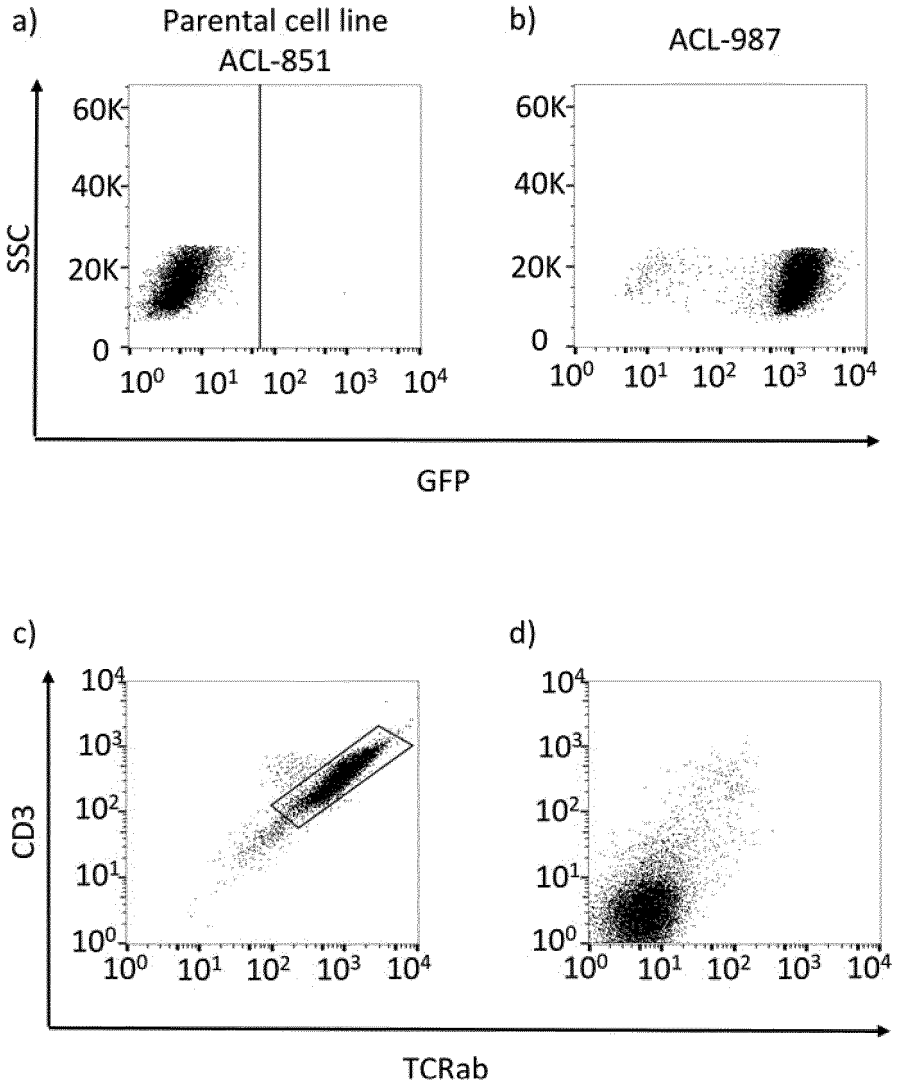

a)
Parental cell line
ACL-851
b)
ACL-987
60K
40K
20K
0
SSC
10^0 10^1 10^2 10^3 10^4
GFP
c)
d)
CD3
TCRab

Figure 50

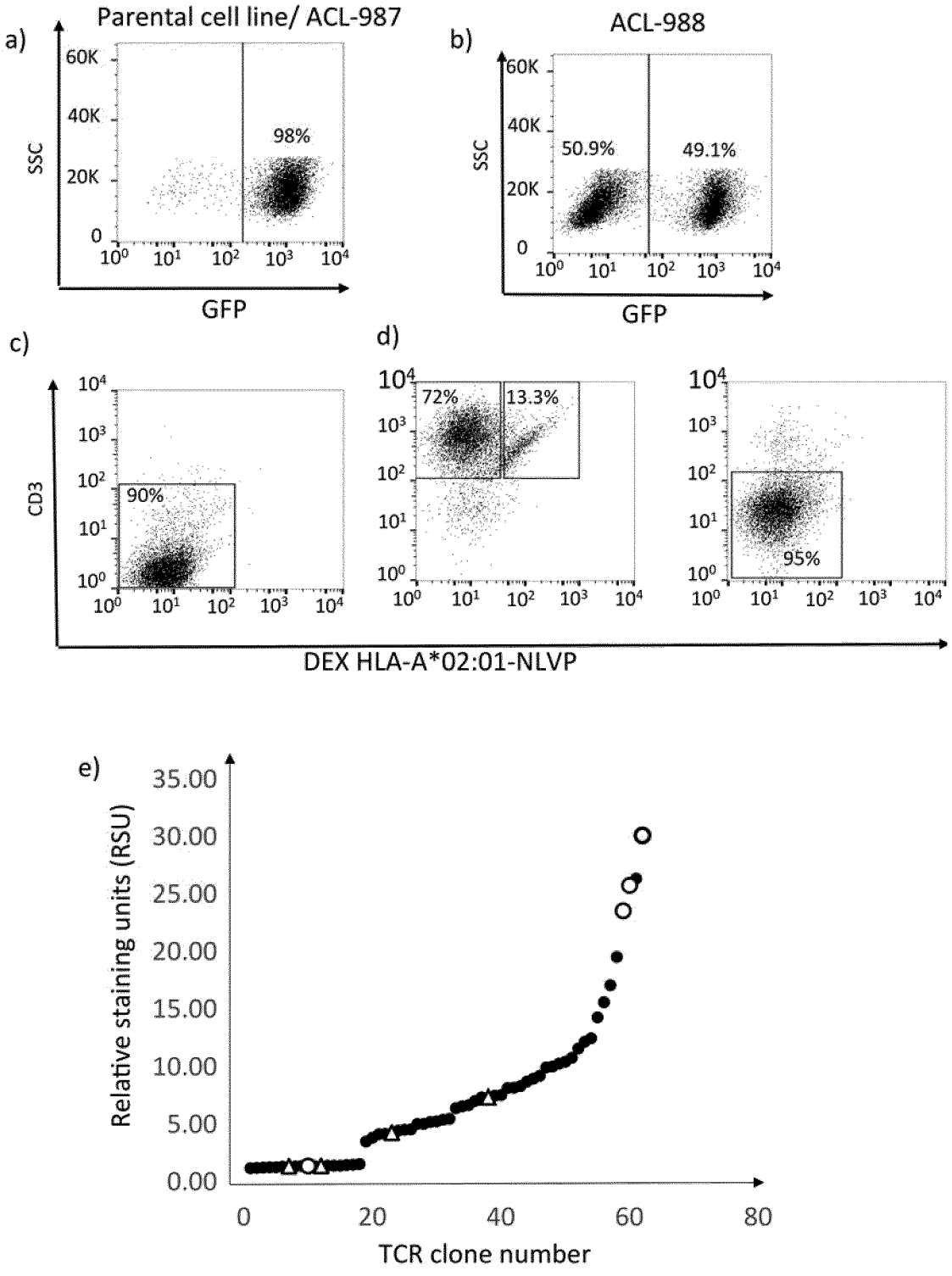

a)
Parental cell line/ ACL-987
SSC
GFP
98%
b)
ACL-988
50.9%
49.1%
c)
CD3
90%
d)
72%
13.3%
95%
DEX HLA-A*02:01-NLVP
e)
Relative staining units (RSU)
TCR clone number

Figure 51

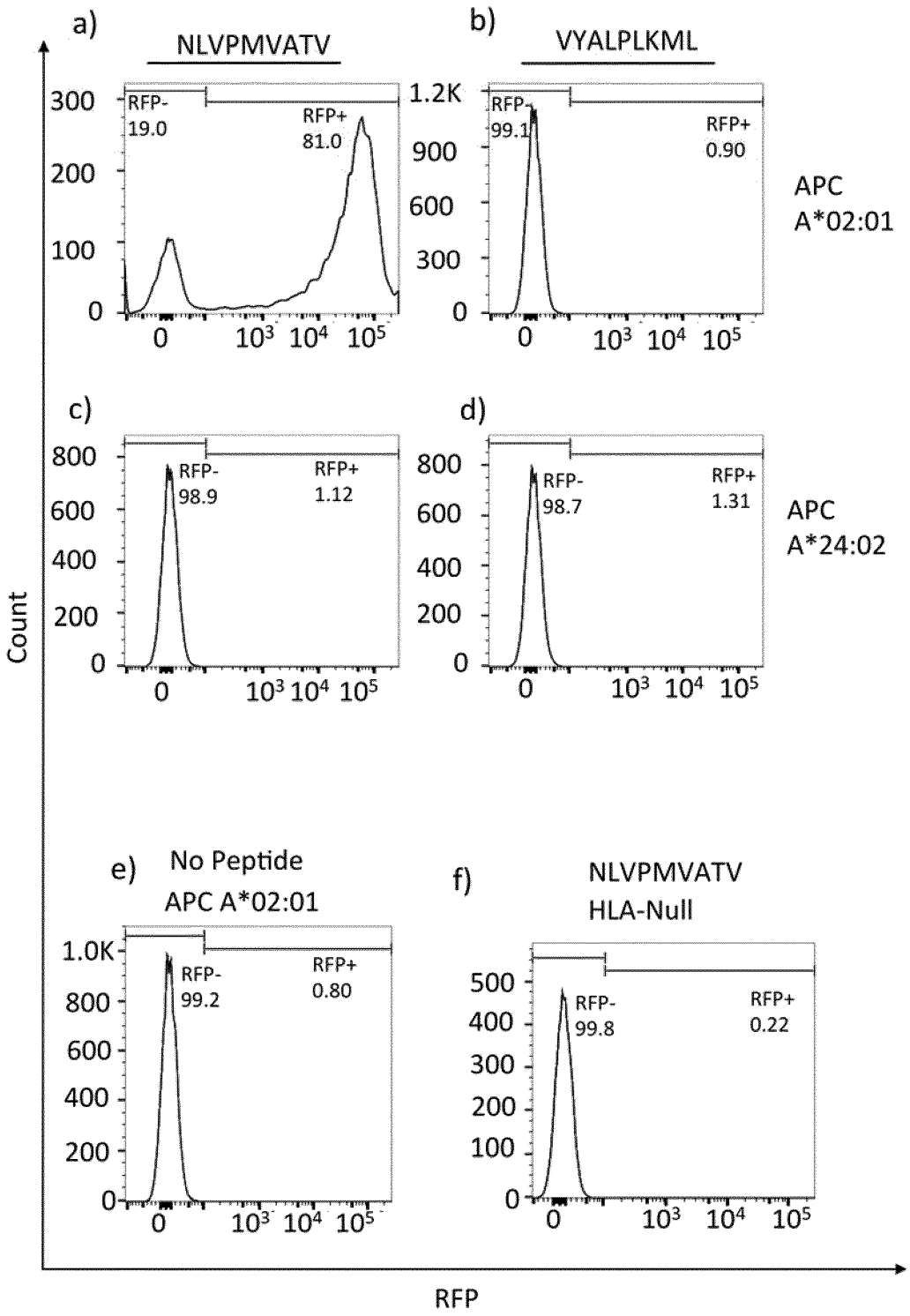
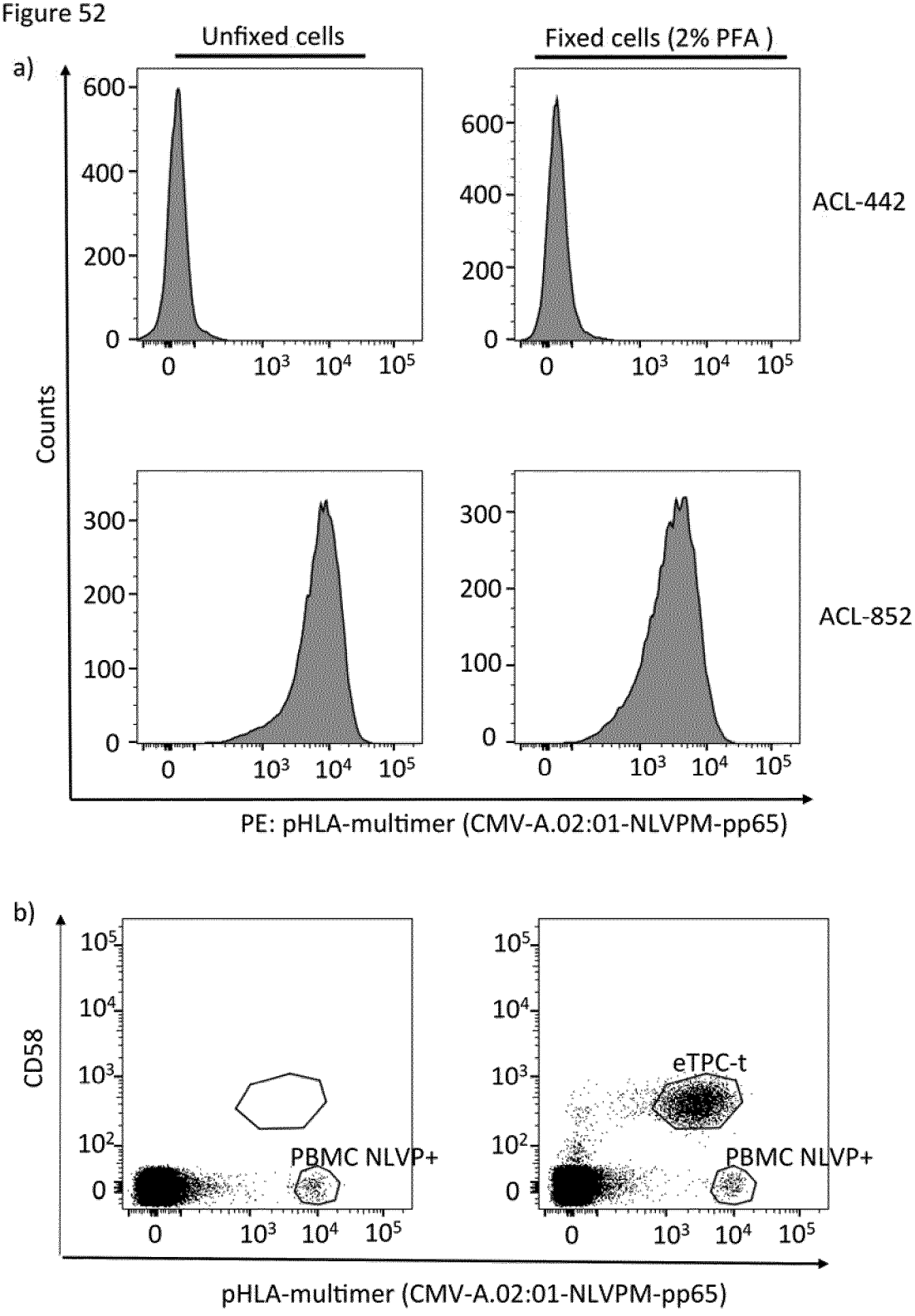

a)
NLVPMVATV
RFP-
19.0
RFP+
81.0
b)
VYALPLKML
RFP-
99.1
RFP+
0.90
APC
A*02:01
c)
RFP-
98.9
RFP+
1.12
d)
RFP-
98.7
RFP+
1.31
APC
A*24:02
Count
e)
No Peptide
APC A*02:01
RFP-
99.2
RFP+
0.80
f)
NLVPMVATV
HLA-Null
RFP-
99.8
RFP+
0.22
RFP

Figure 52


a)
Unfixed cells
Fixed cells (2% PFA)
ACL-442
ACL-852
Counts
PE: pHLA-multimer (CMV-A.02:01-NLVPM-pp65)
b)
CD58
PBMC NLVP+
eTPC-t
pHLA-multimer (CMV-A.02:01-NLVPM-pp65)