Figure 1

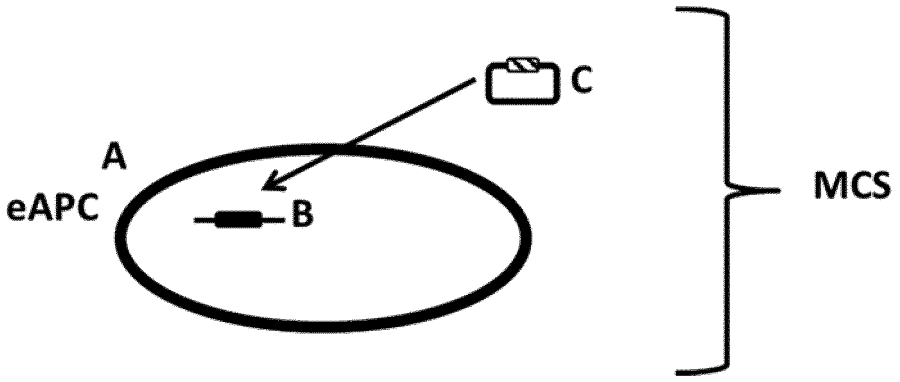


Figure 2

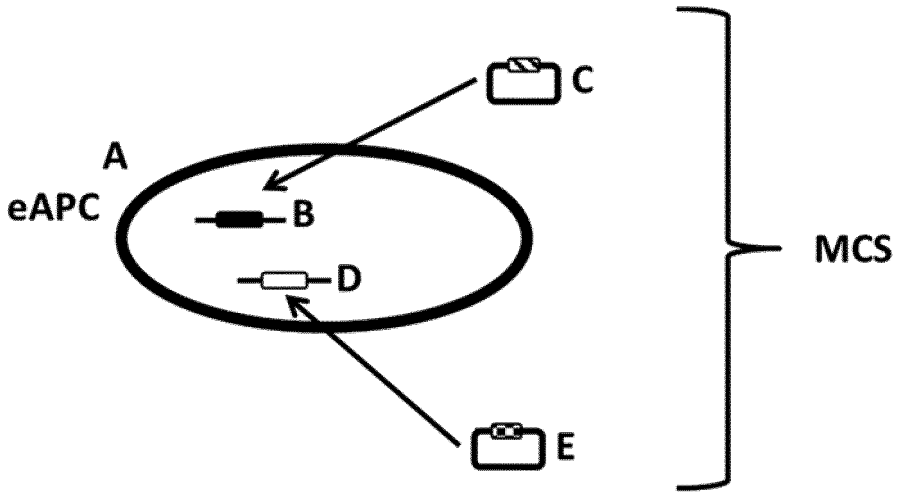


Figure 3

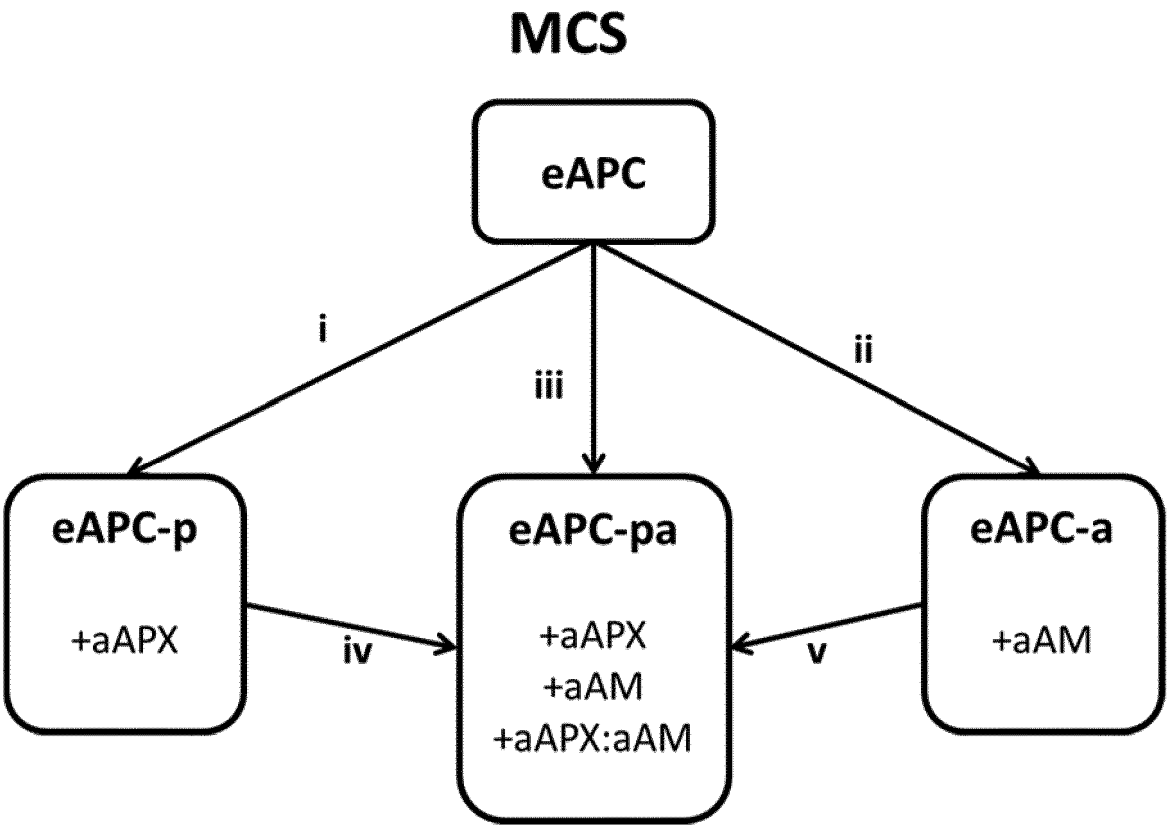

MCS
eAPC
i
iii
ii
eAPC-p
+aAPX
iv
eAPC-pa
+aAPX
+aAM
+aAPX:aAM
v
eAPC-a
+aAM

Figure 4

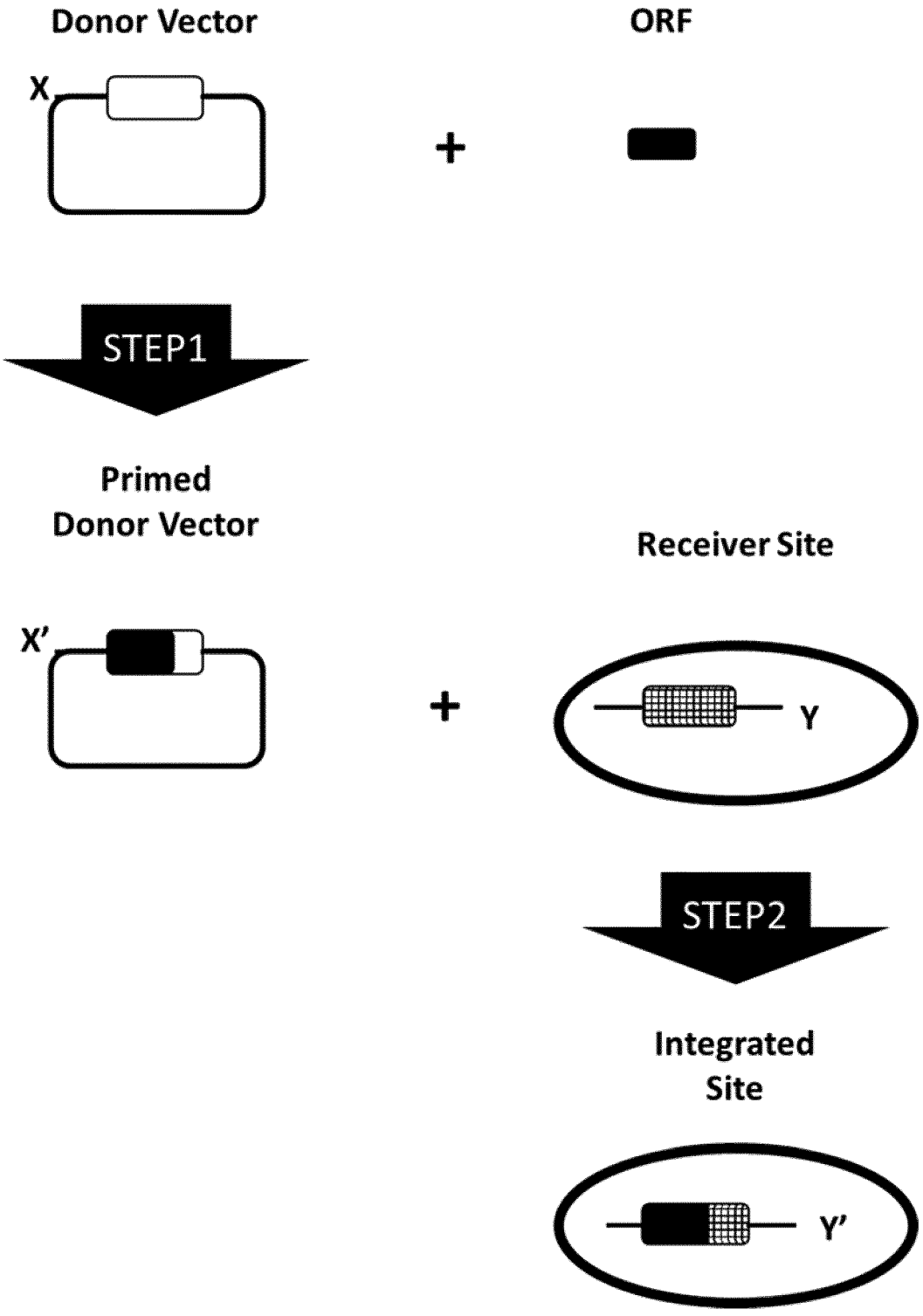

Donor Vector
X
+
ORF
STEP1
Primed
Donor Vector
X'
+
Receiver Site
Y
STEP2
Integrated
Site
Y'

Figure 5

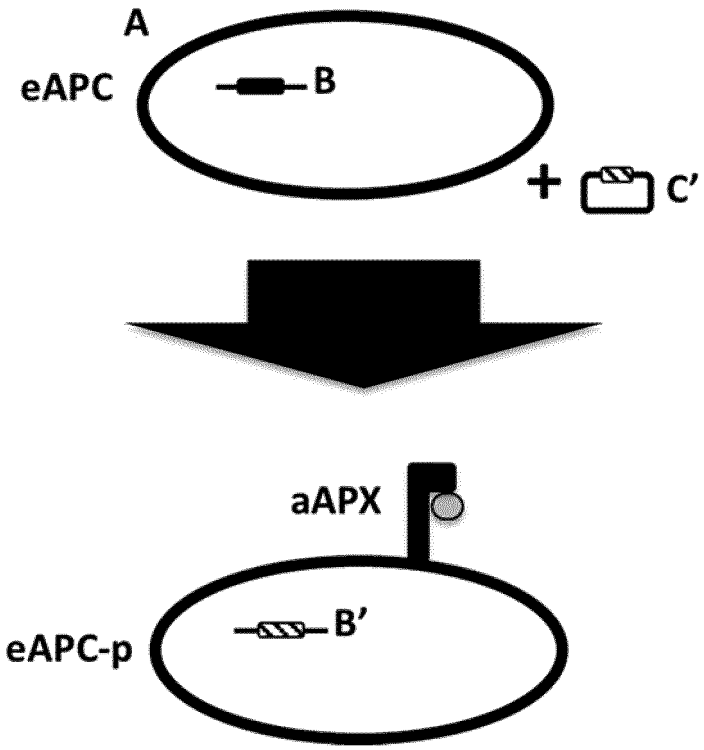


Figure 6

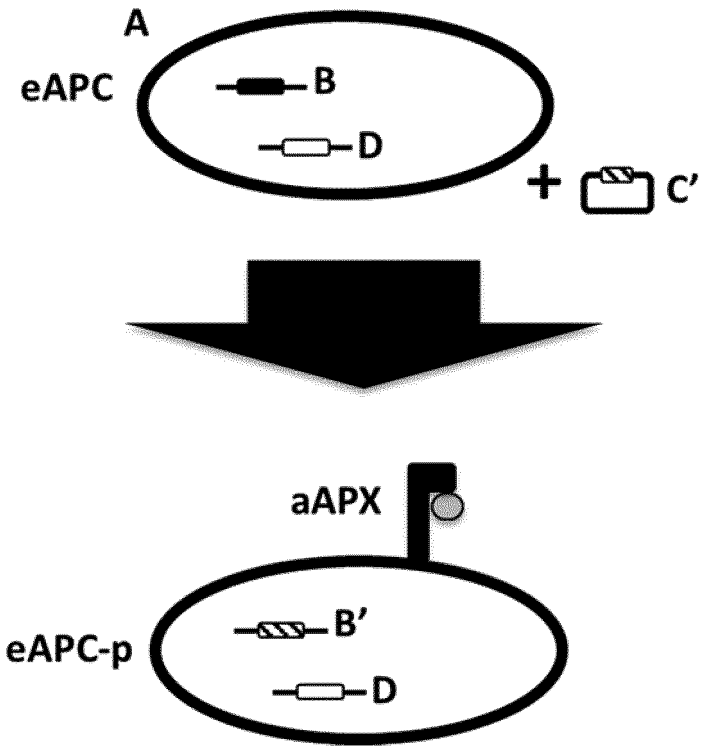


Figure 7

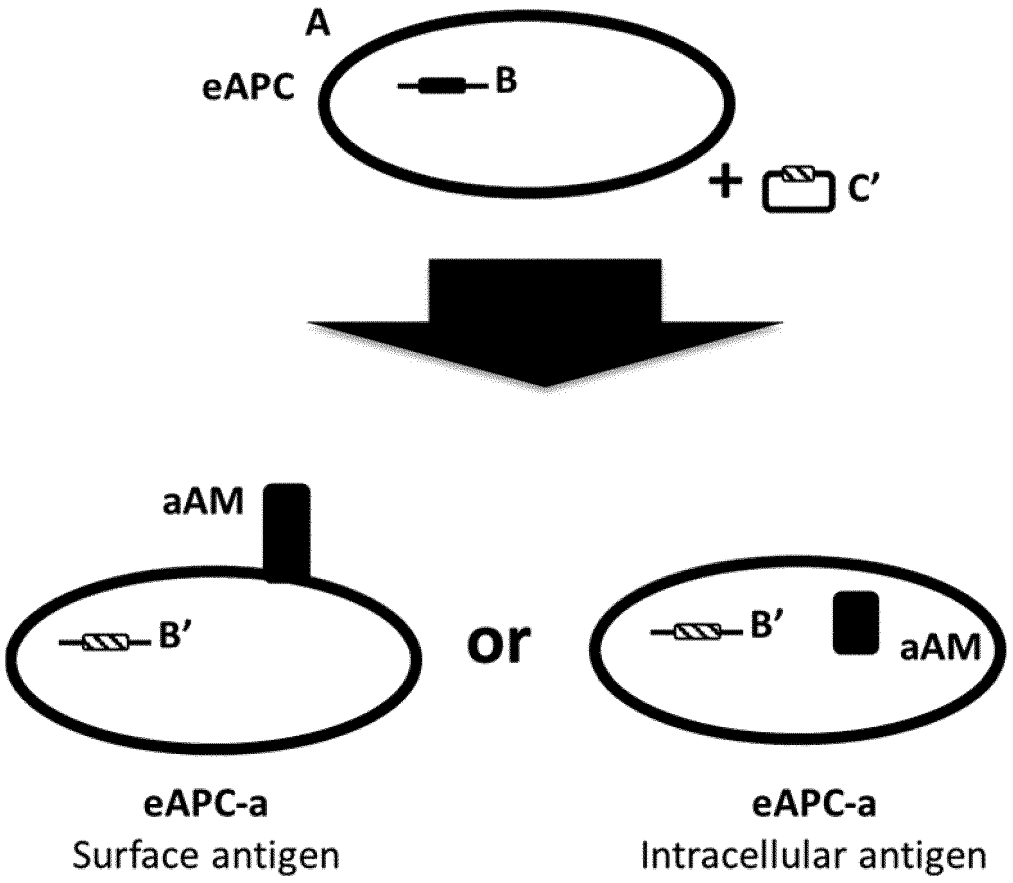

A
eAPC
B
+
C'
aAM
B'
or
B'
aAM
eAPC-a
Surface antigen
eAPC-a
Intracellular antigen

Figure 8

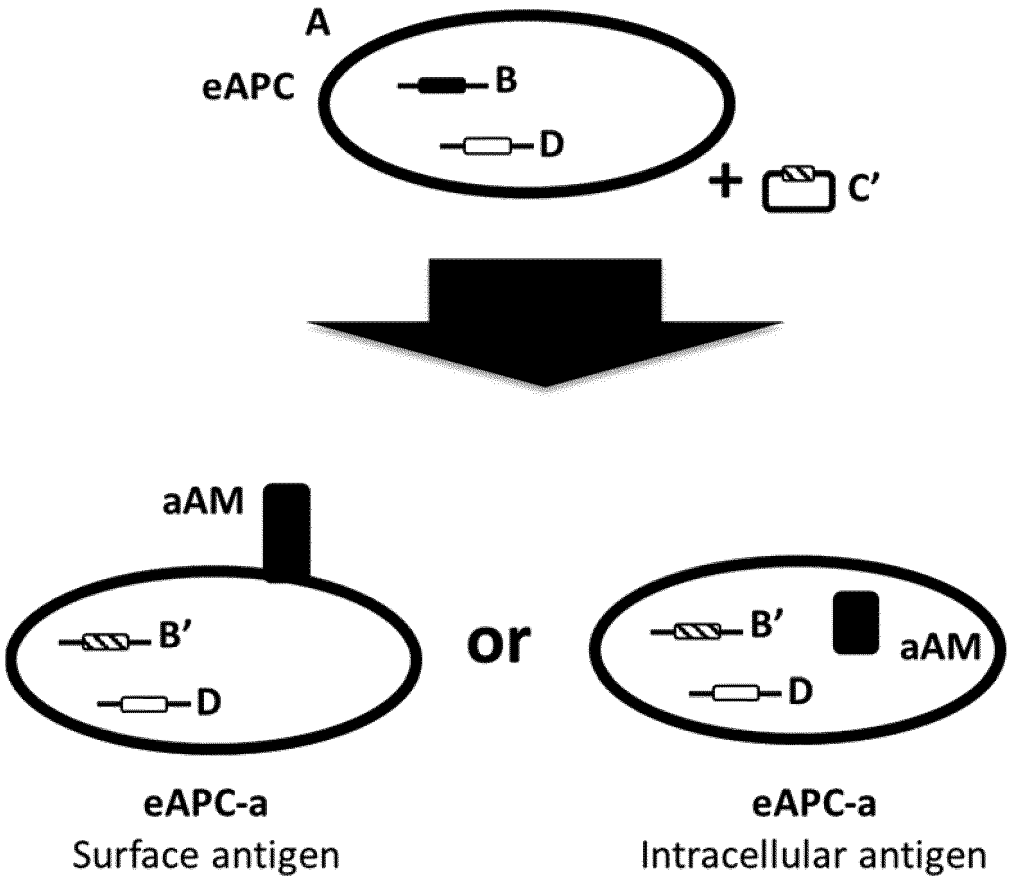

A
eAPC
B
D
+
C'
aAM
B'
D
or
B'
aAM
D
eAPC-a
Surface antigen
eAPC-a
Intracellular antigen

Figure 9

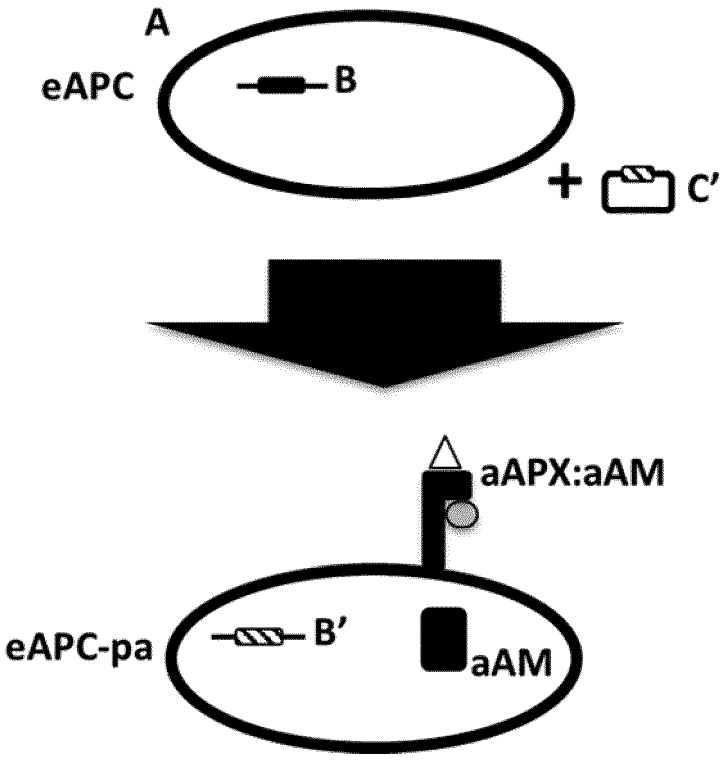


Figure 10

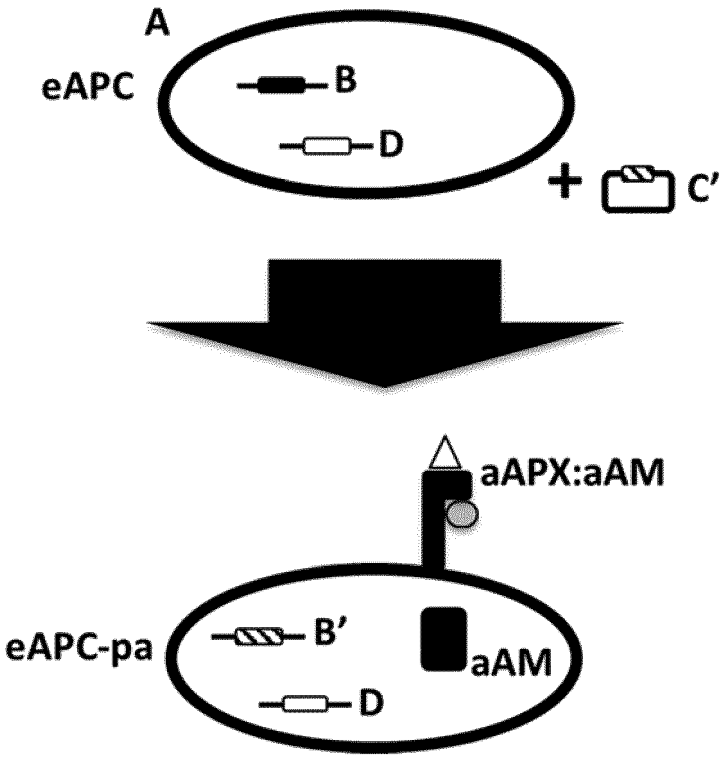


Figure 11

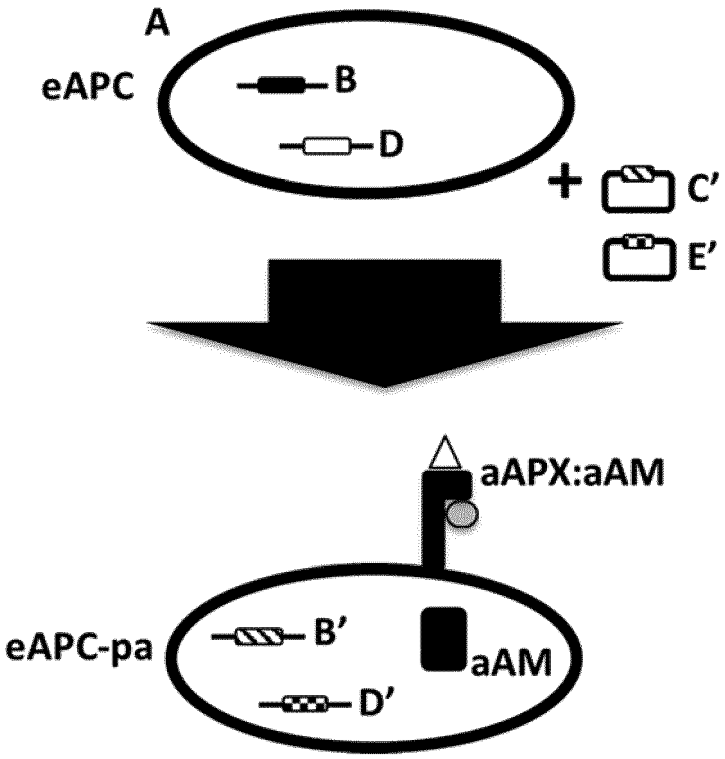


Figure 12

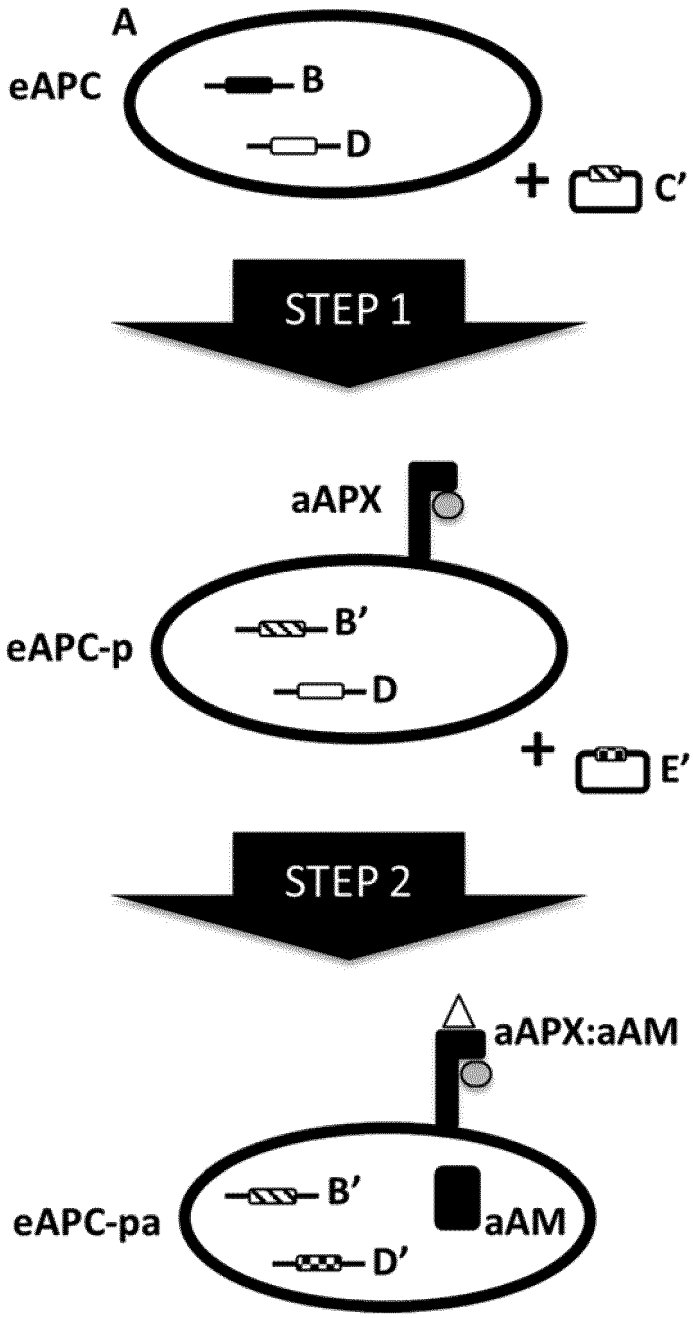


Figure 13

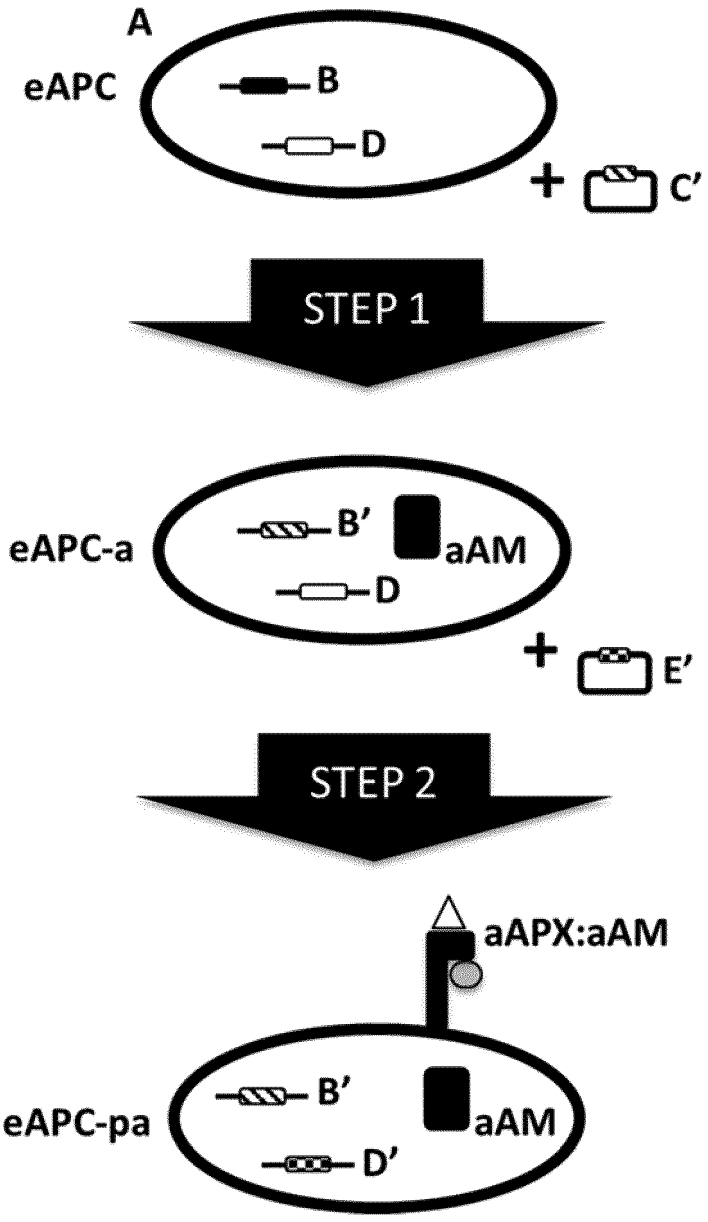


Figure 14

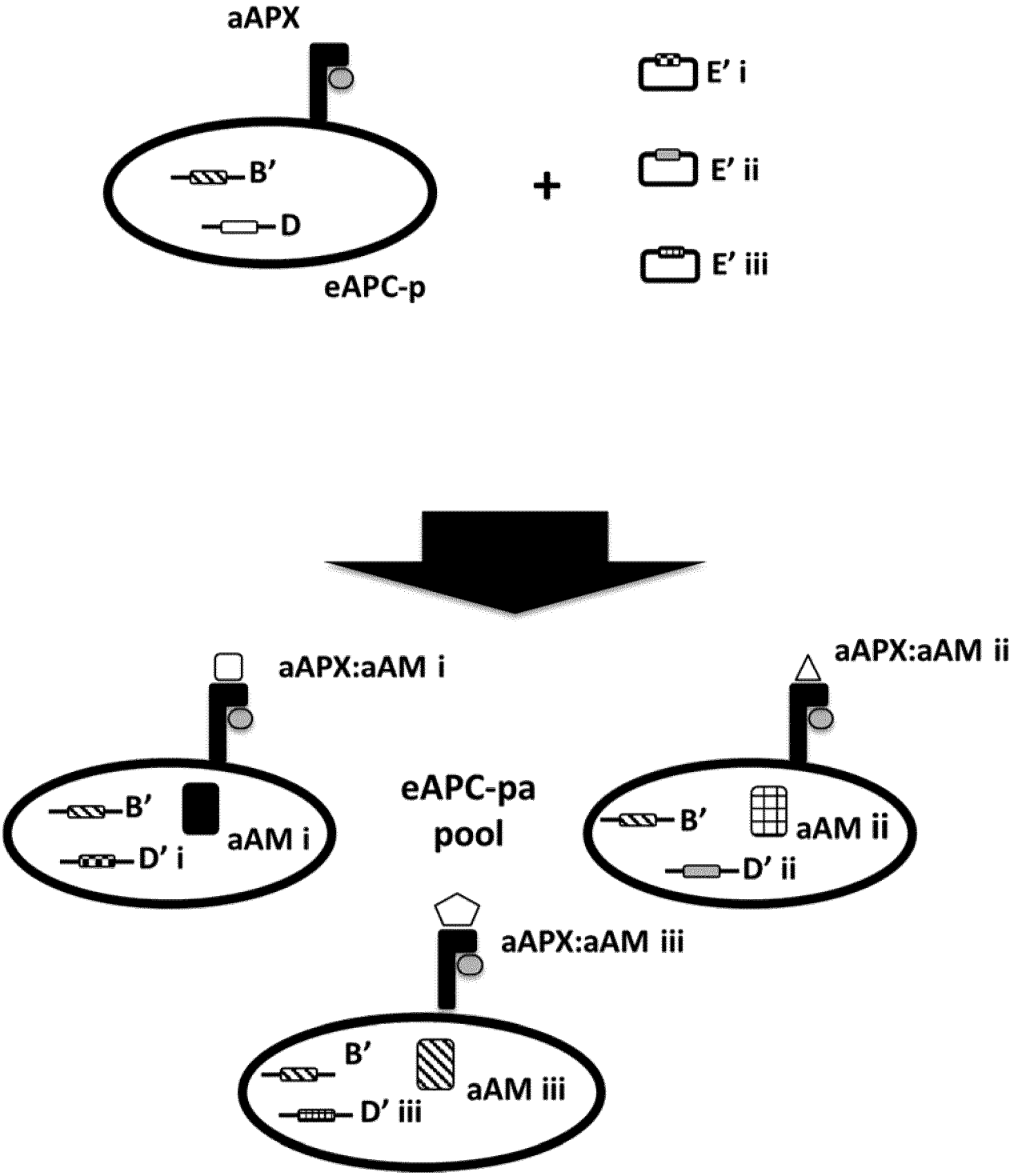

aAPX
B'
D
eAPC-p
+
E' i
E' ii
E' iii
aAPX:aAM i
B'
D' i
aAM i
eAPC-pa pool
aAPX:aAM ii
B'
aAM ii
D' ii
aAPX:aAM iii
B'
D' iii
aAM iii

Figure 15

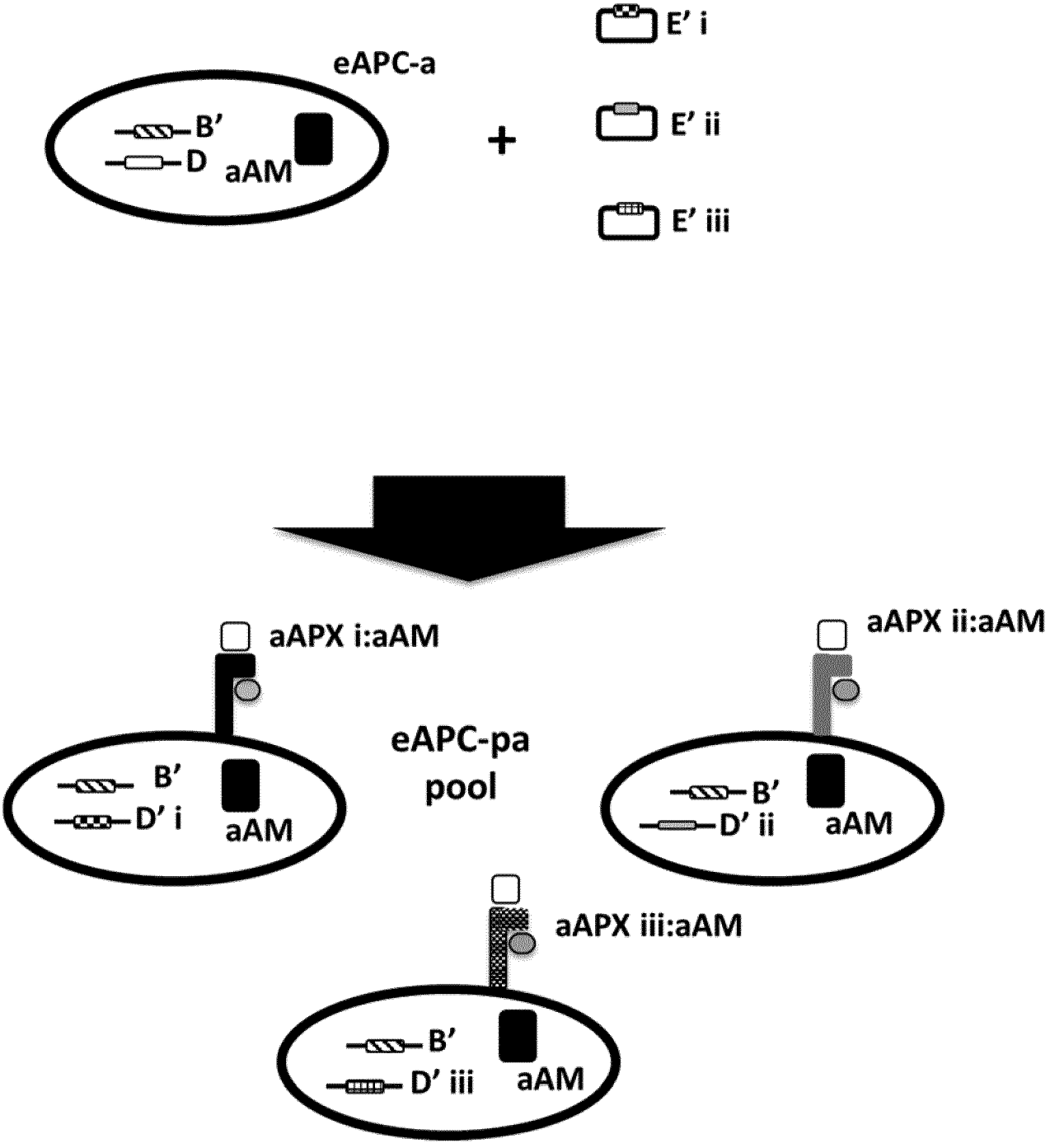

eAPC-a
B'
D
aAM
+
E' i
E' ii
E' iii
aAPX i:aAM
B'
D' i
aAM
eAPC-pa
pool
aAPX ii:aAM
B'
D' ii
aAM
aAPX iii:aAM
B'
D' iii
aAM

Figure 16

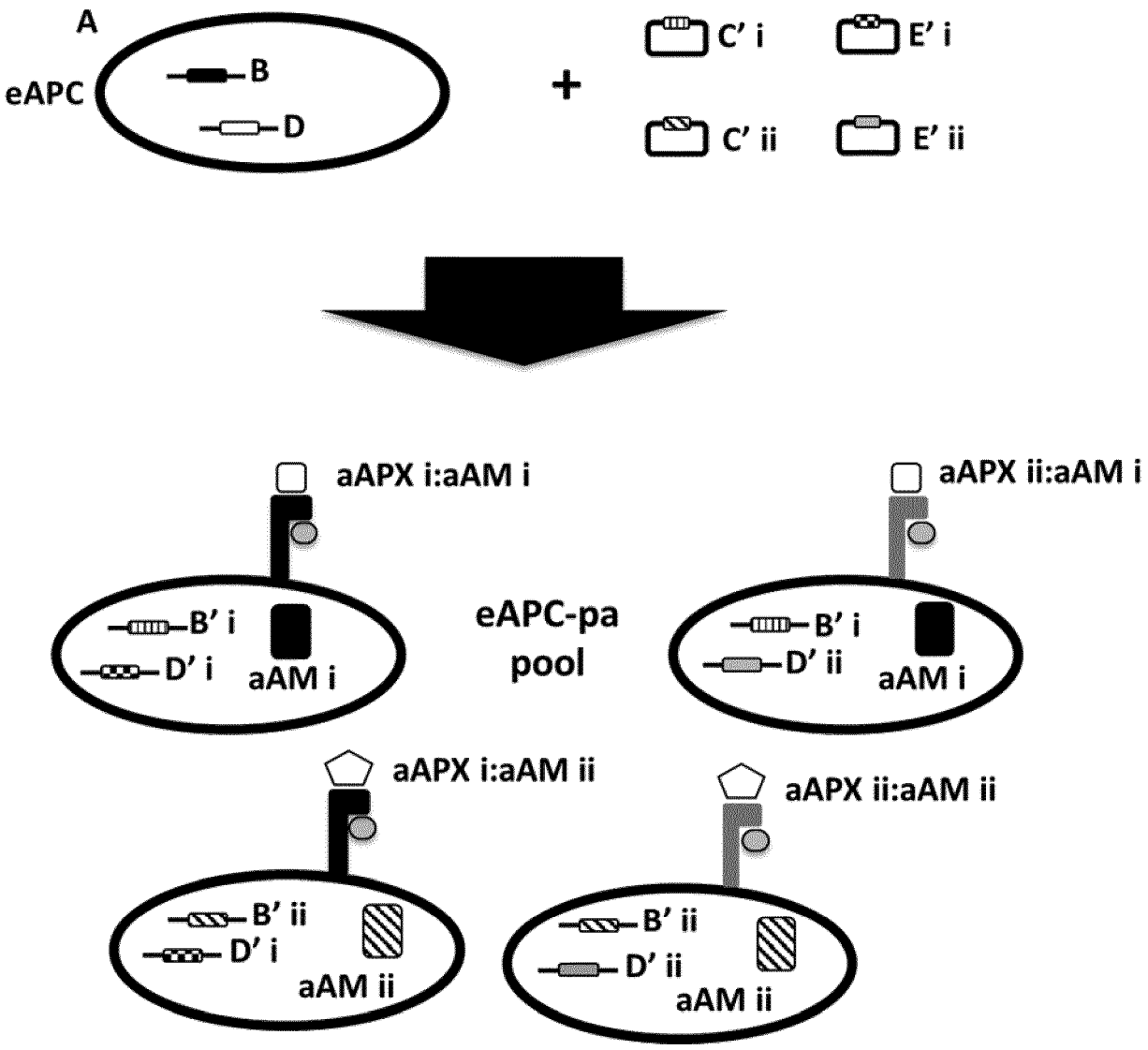

A
eAPC
B
D
+
C' i
E' i
C' ii
E' ii
aAPX i:aAM i
B' i
D' i
aAM i
eAPC-pa
pool
aAPX ii:aAM i
B' i
D' ii
aAM i
aAPX i:aAM ii
B' ii
D' i
aAM ii
aAPX ii:aAM ii
B' ii
D' ii
aAM ii

Figure 17

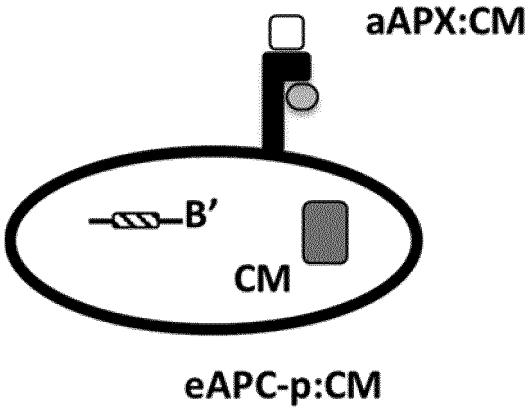


Figure 18

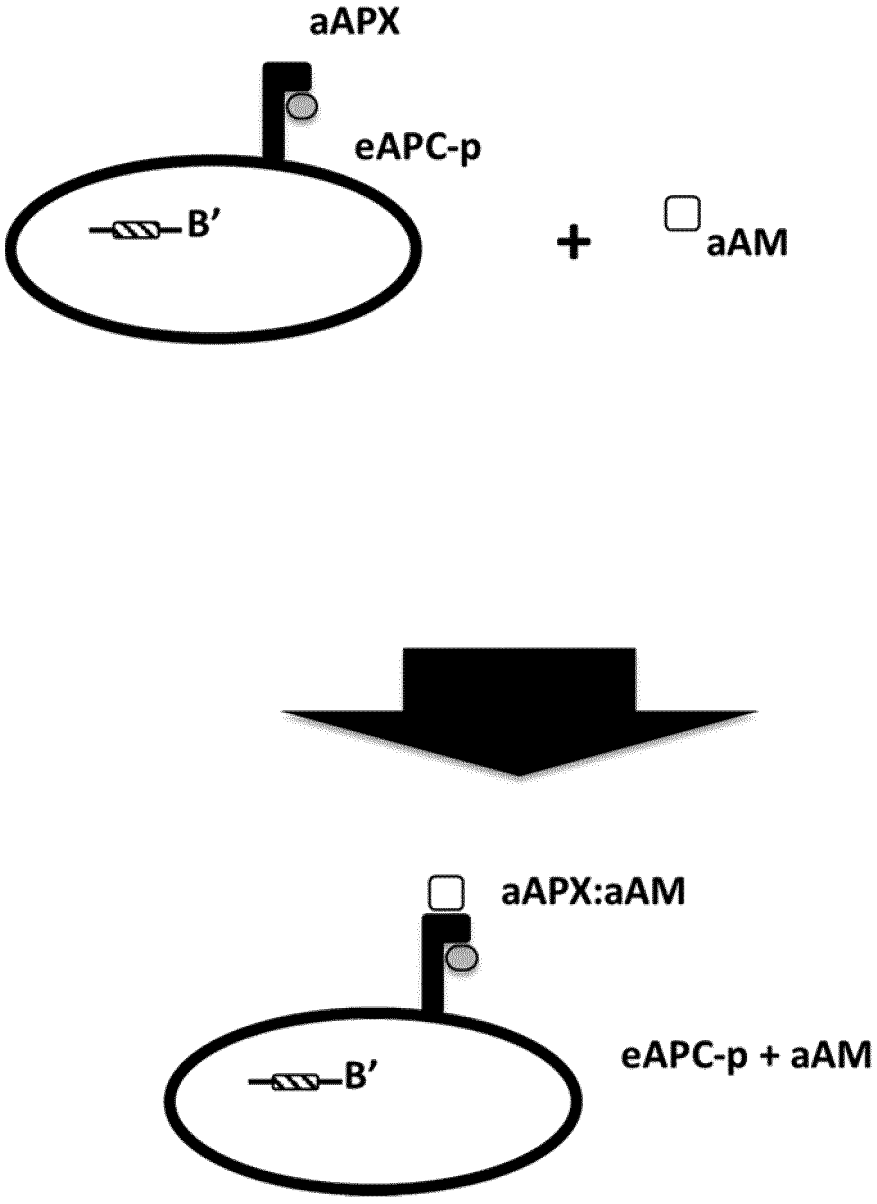


Figure 19

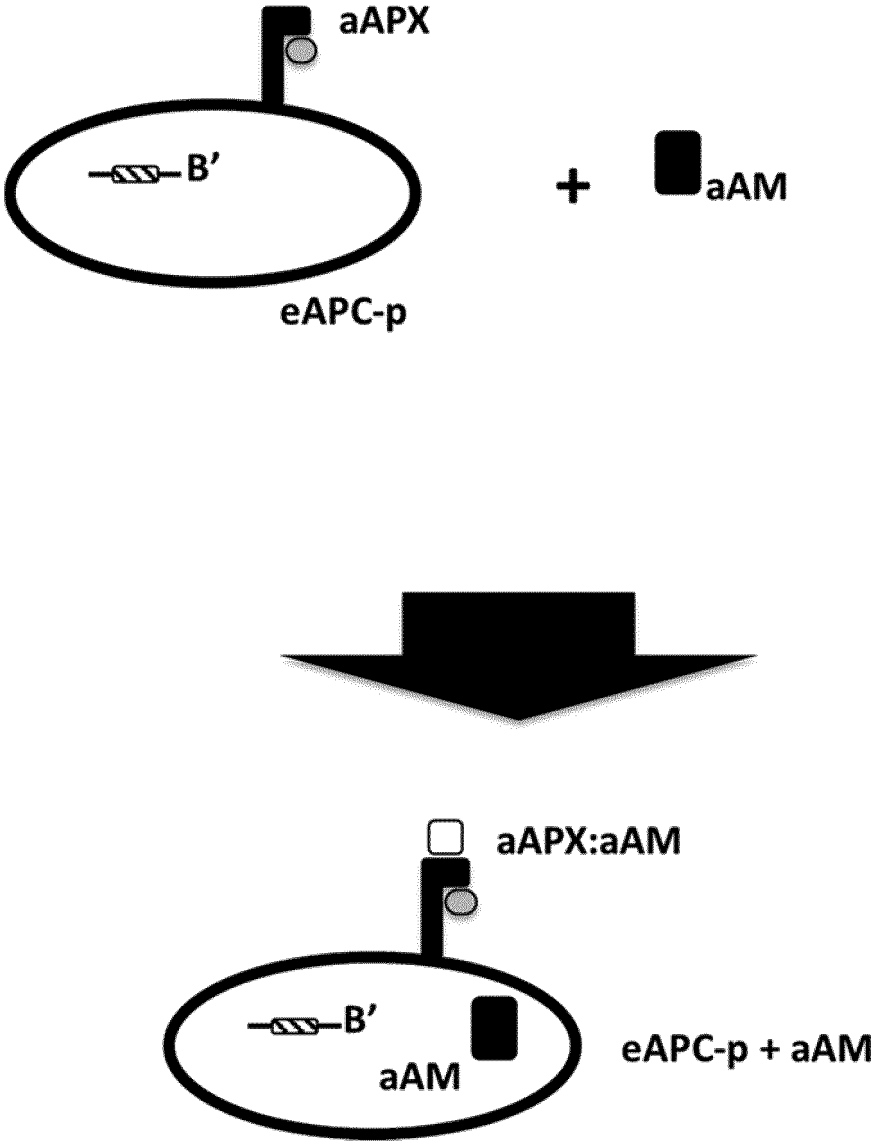


Figure 20

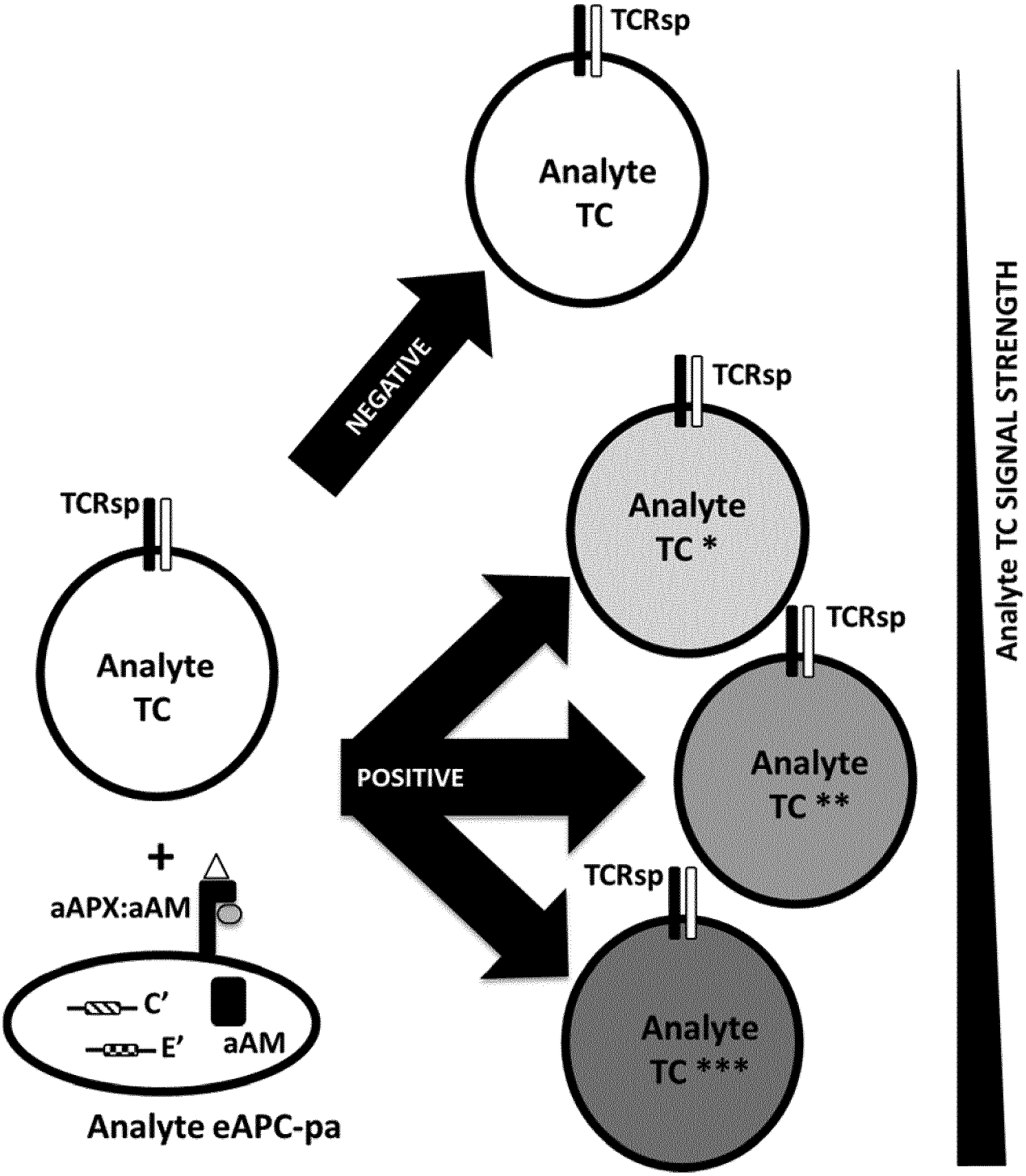

TCRsp
Analyte TC
NEGATIVE
TCRsp
Analyte TC *
TCRsp
Analyte TC **
TCRsp
Analyte TC ***
POSITIVE
TCRsp
Analyte TC
+
aAPX:aAM
C'
E'
aAM
Analyte eAPC-pa
Analyte TC SIGNAL STRENGTH

Figure 21

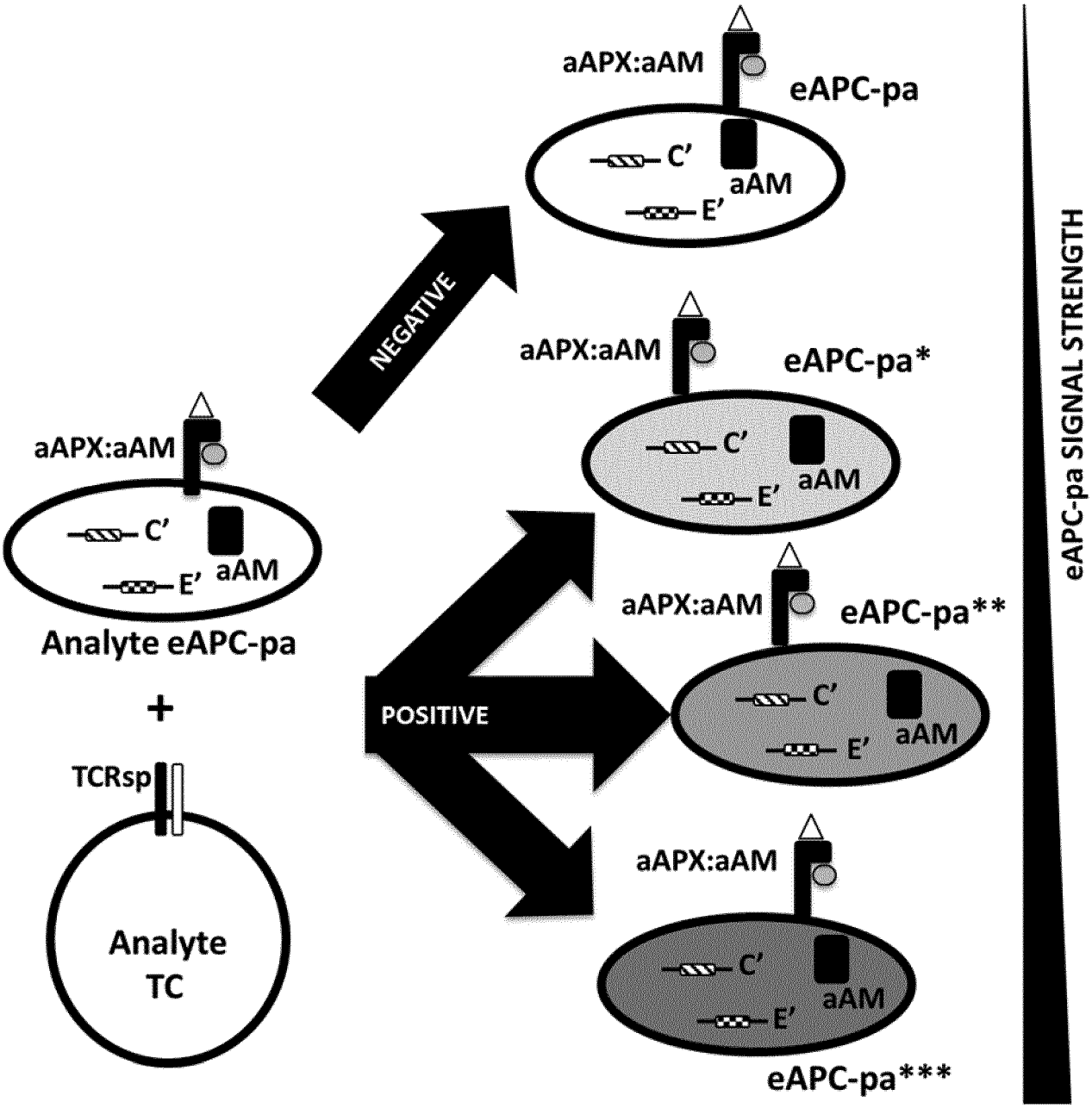

aAPX:aAM
eAPC-pa
C'
aAM
E'
NEGATIVE
aAPX:aAM
eAPC-pa*
C'
aAM
E'
aAPX:aAM
aAM
C'
E'
Analyte eAPC-pa
+
TCRsp
POSITIVE
aAPX:aAM
eAPC-pa**
C'
aAM
E'
Analyte
TC
aAPX:aAM
C'
aAM
E'
eAPC-pa***
eAPC-pa SIGNAL STRENGTH

Figure 22

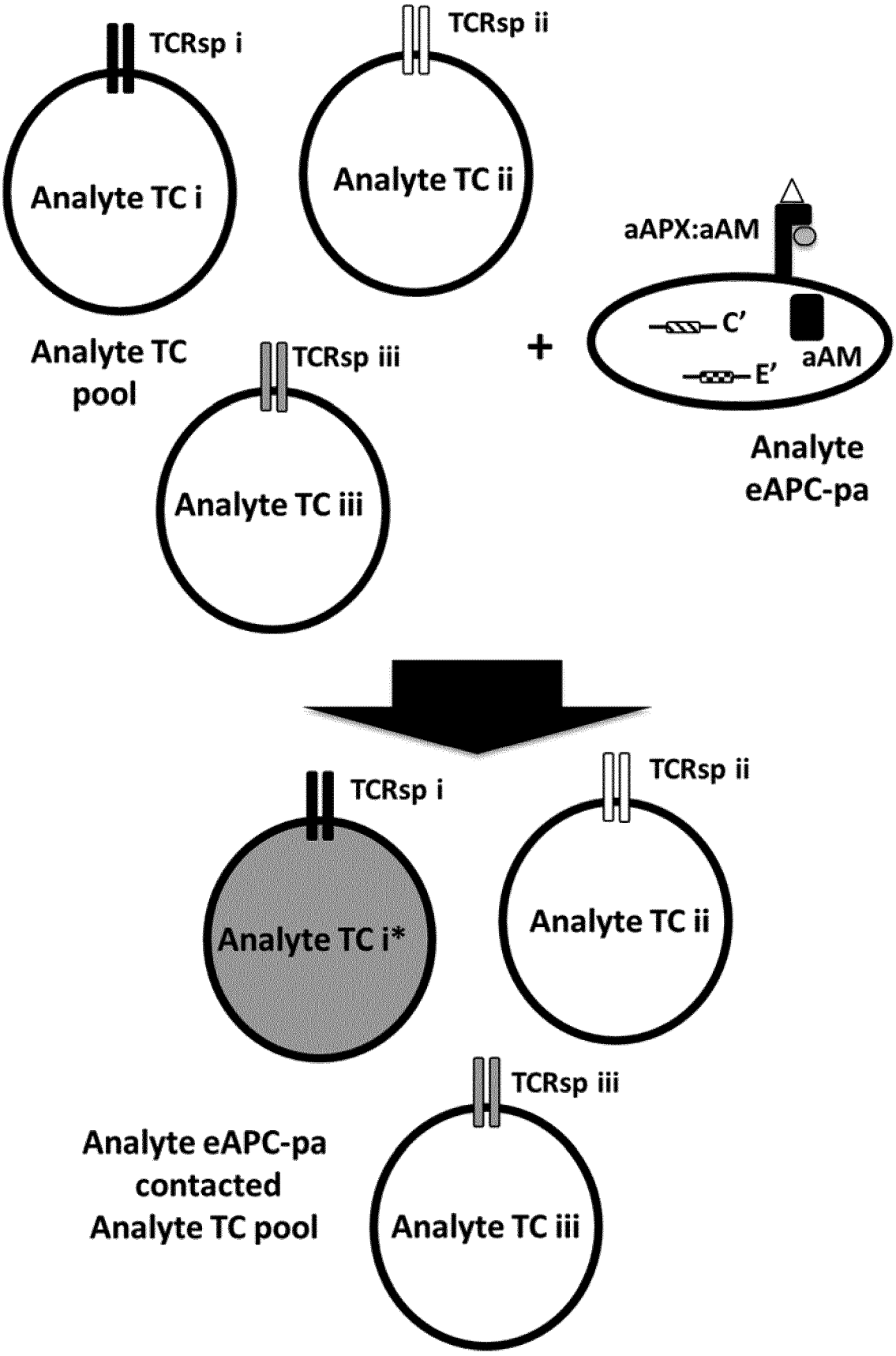

TCRsp i
Analyte TC i
TCRsp ii
Analyte TC ii
Analyte TC pool
TCRsp iii
Analyte TC iii
+
aAPX:aAM
C'
E'
aAM
Analyte eAPC-pa
TCRsp i
Analyte TC i*
TCRsp ii
Analyte TC ii
TCRsp iii
Analyte TC iii
Analyte eAPC-pa contacted Analyte TC pool

Figure 23

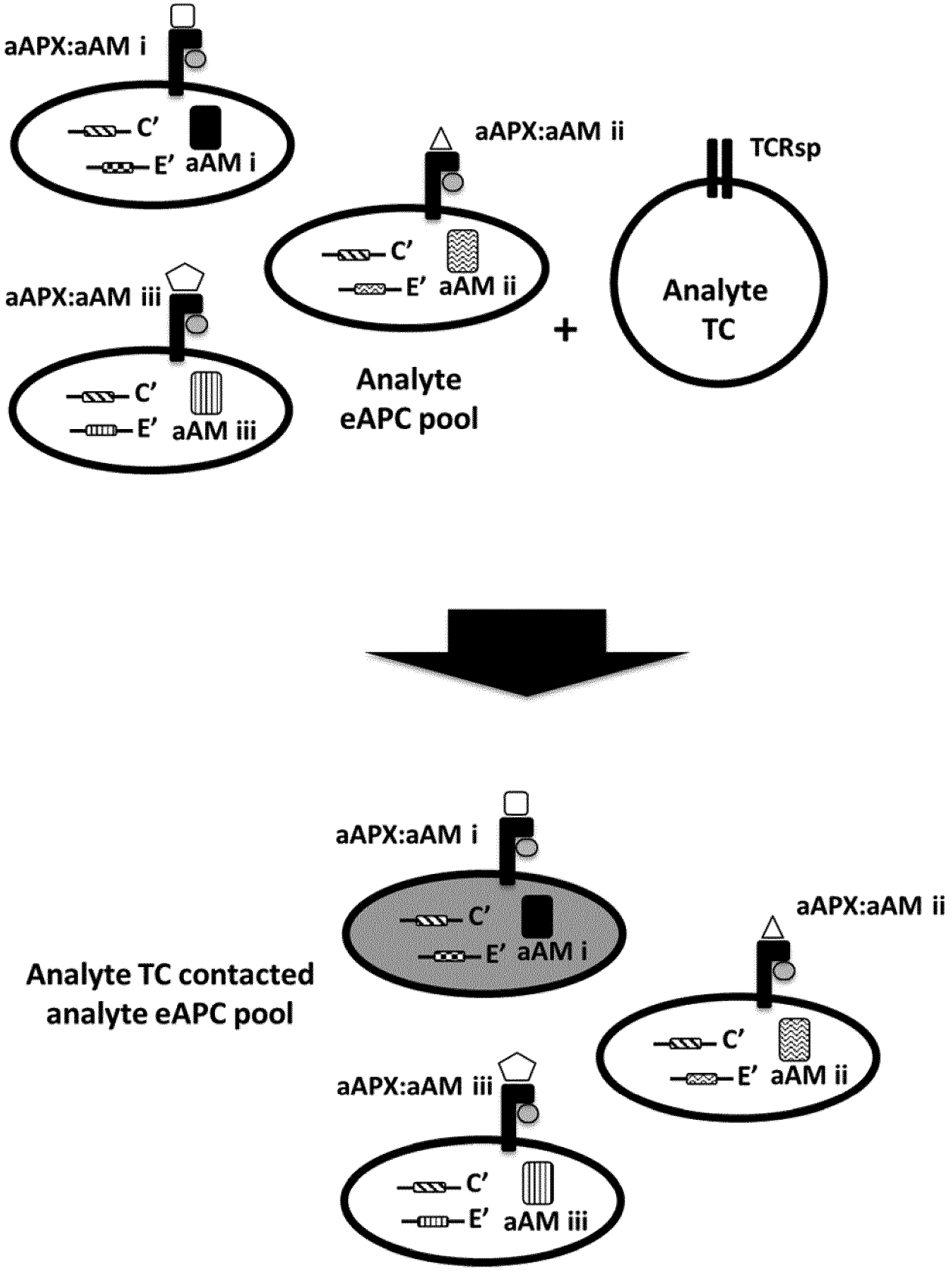

aAPX:aAM i
C'
E'
aAM i
aAPX:aAM ii
C'
E'
aAM ii
TCRsp
Analyte
TC
+
aAPX:aAM iii
C'
E'
aAM iii
Analyte
eAPC pool
aAPX:aAM i
C'
E'
aAM i
aAPX:aAM ii
C'
E'
aAM ii
Analyte TC contacted
analyte eAPC pool
aAPX:aAM iii
C'
E'
aAM iii

Figure 24

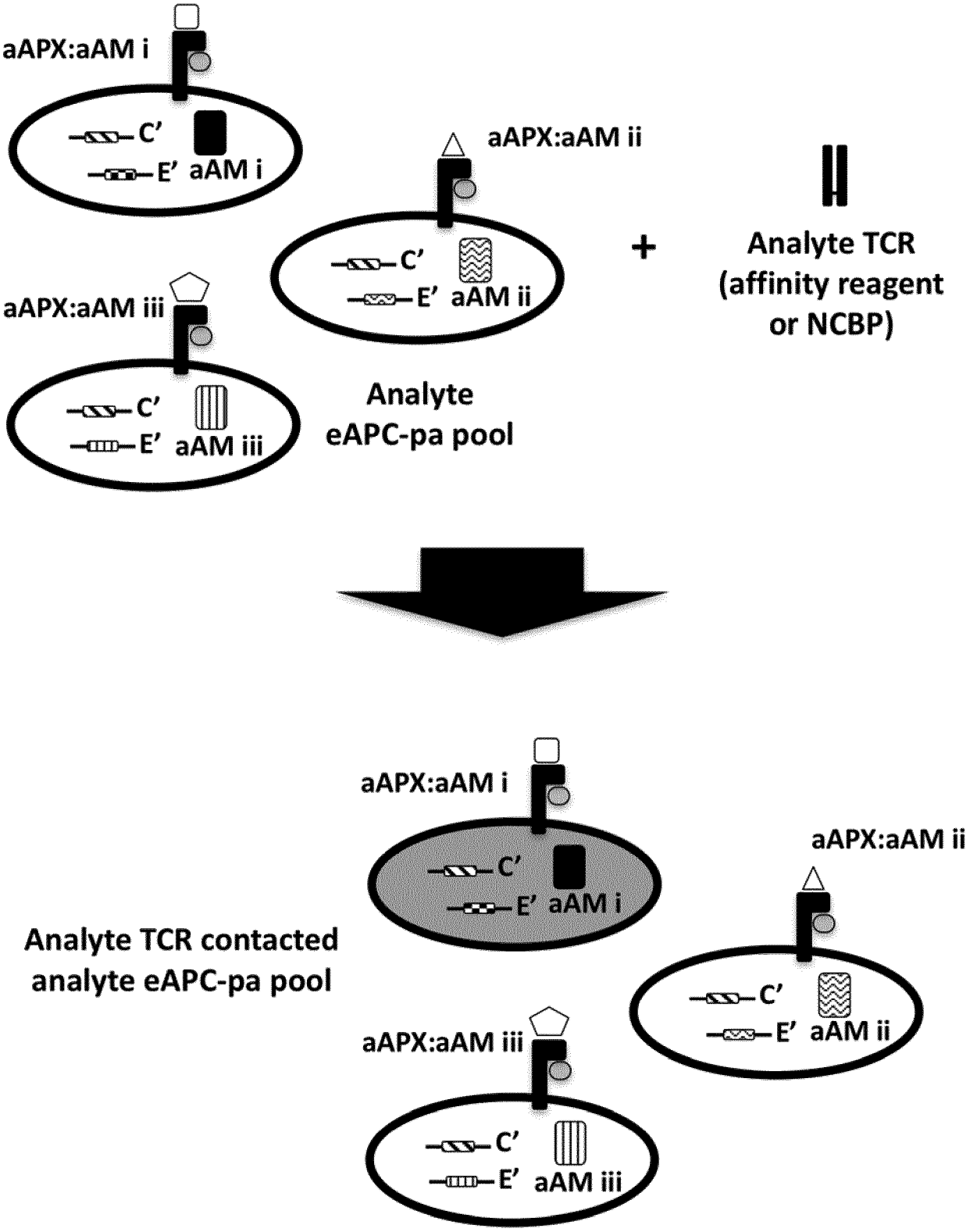

aAPX:aAM i
C'
E' aAM i
aAPX:aAM ii
C'
E' aAM ii
+
Analyte TCR
(affinity reagent
or NCBP)
aAPX:aAM iii
C'
E' aAM iii
Analyte
eAPC-pa pool
aAPX:aAM i
C'
E' aAM i
aAPX:aAM ii
C'
E' aAM ii
Analyte TCR contacted
analyte eAPC-pa pool
aAPX:aAM iii
C'
E' aAM iii

Figure 25

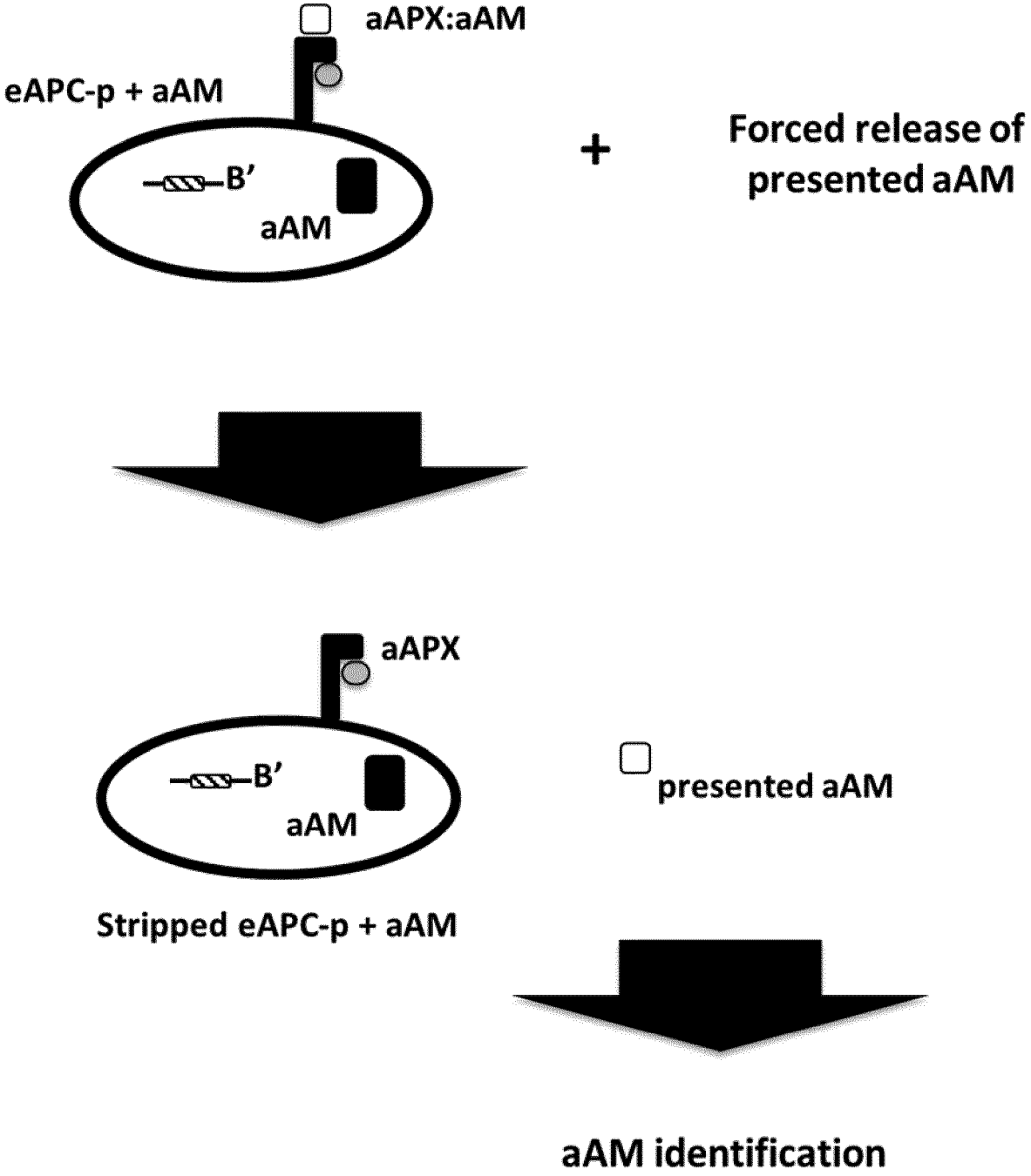

aAPX:aAM
eAPC-p + aAM
B'
aAM
+
Forced release of presented aAM
aAPX
B'
aAM
presented aAM
Stripped eAPC-p + aAM
aAM identification

Figure 26

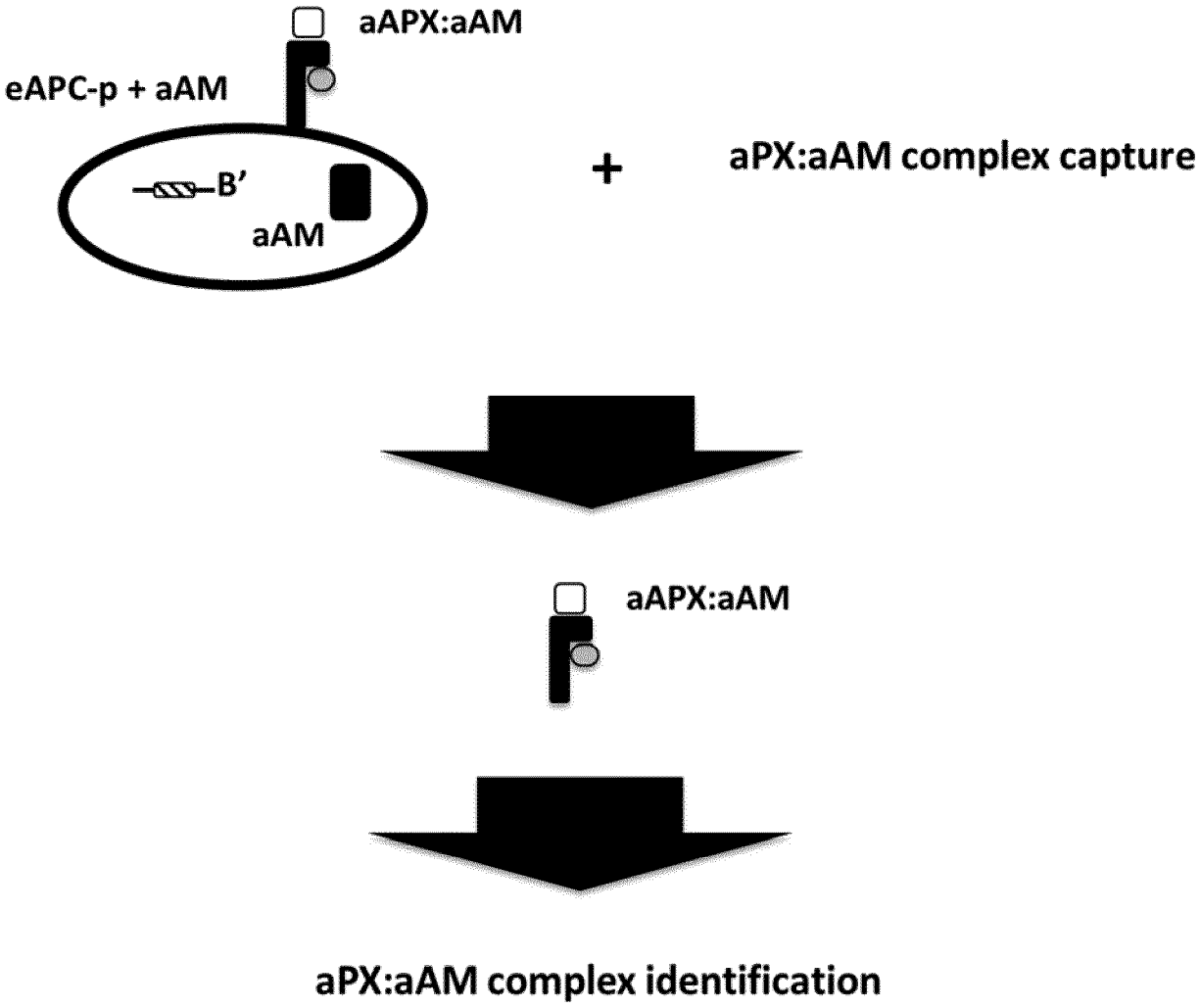

aAPX:aAM
eAPC-p + aAM
B'
aAM
+
aPX:aAM complex capture
aAPX:aAM
aPX:aAM complex identification

Figure 27

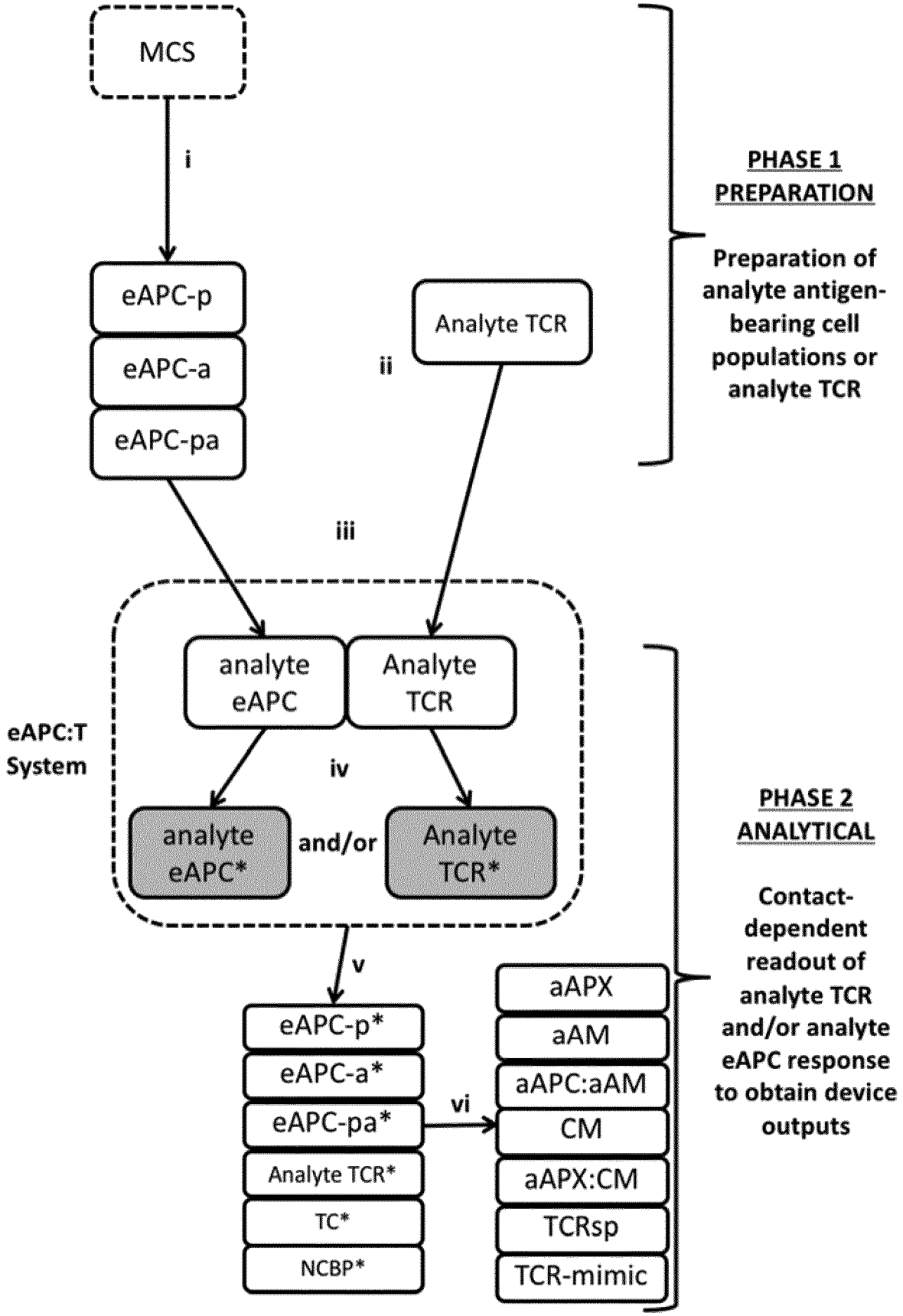

MCS
i
eAPC-p
eAPC-a
eAPC-pa
ii
Analyte TCR
PHASE 1
PREPARATION
Preparation of analyte antigen-bearing cell populations or analyte TCR
iii
eAPC:T System
analyte eAPC
Analyte TCR
iv
analyte eAPC*
and/or
Analyte TCR*
v
eAPC-p*
eAPC-a*
eAPC-pa*
Analyte TCR*
TC*
NCBP*
vi
aAPX
aAM
aAPC:aAM
CM
aAPX:CM
TCRsp
TCR-mimic
PHASE 2
ANALYTICAL
Contact-dependent readout of analyte TCR and/or analyte eAPC response to obtain device outputs

Figure 28

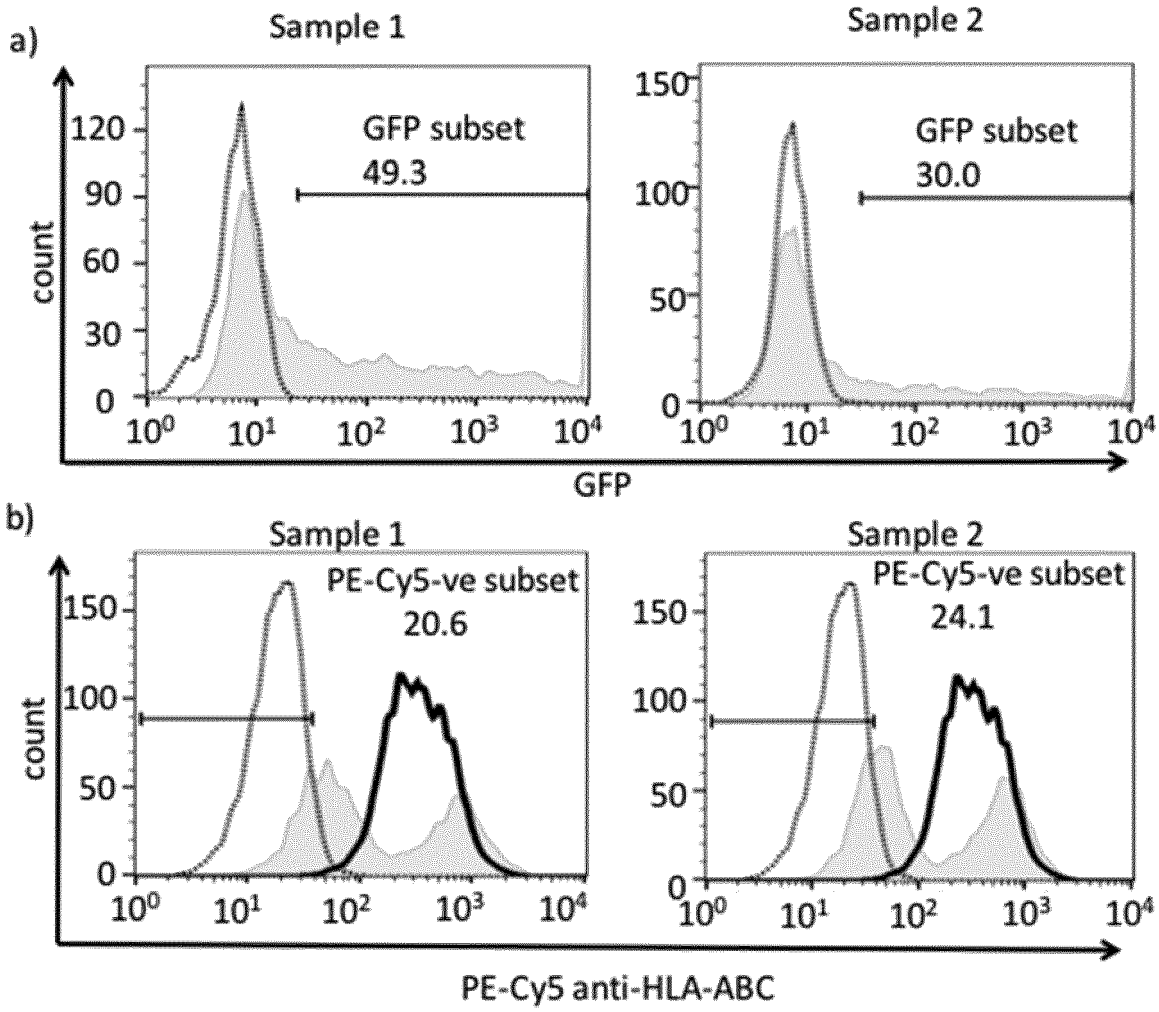
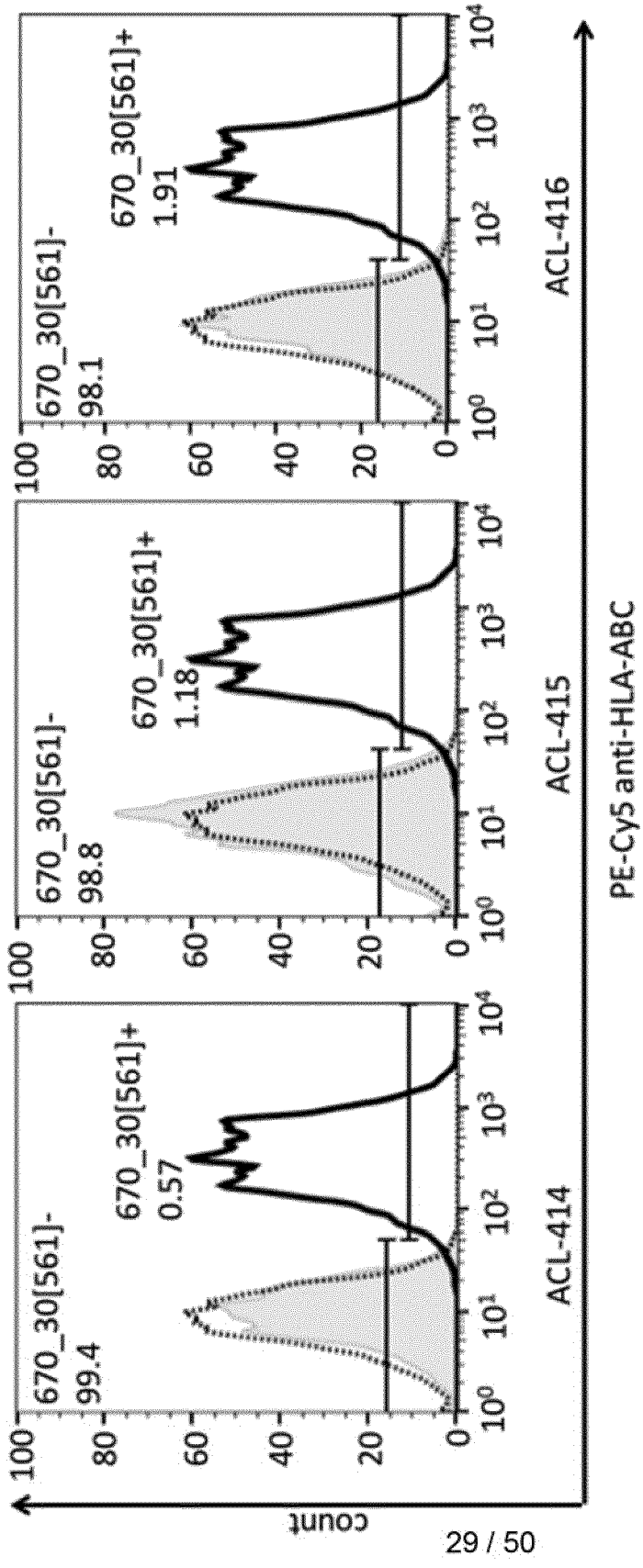

a)
Sample 1
Sample 2
GFP subset
49.3
GFP subset
30.0
count
GFP
b)
Sample 1
Sample 2
PE-Cy5-ve subset
20.6
PE-Cy5-ve subset
24.1
count
PE-Cy5 anti-HLA-ABC

Figure 29


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
count
ACL-414
ACL-415
ACL-416
PE-Cy5 anti-HLA-ABC

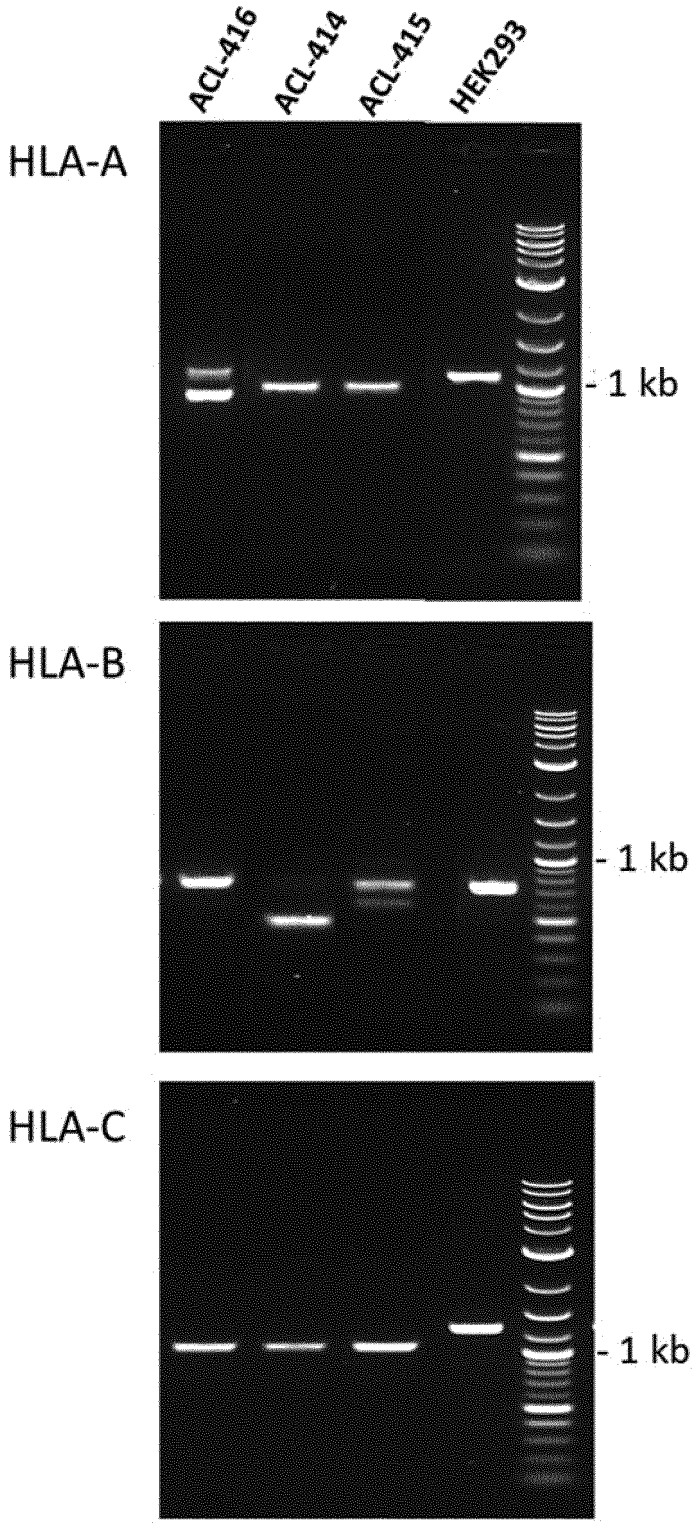


Figure 30

Figure 31

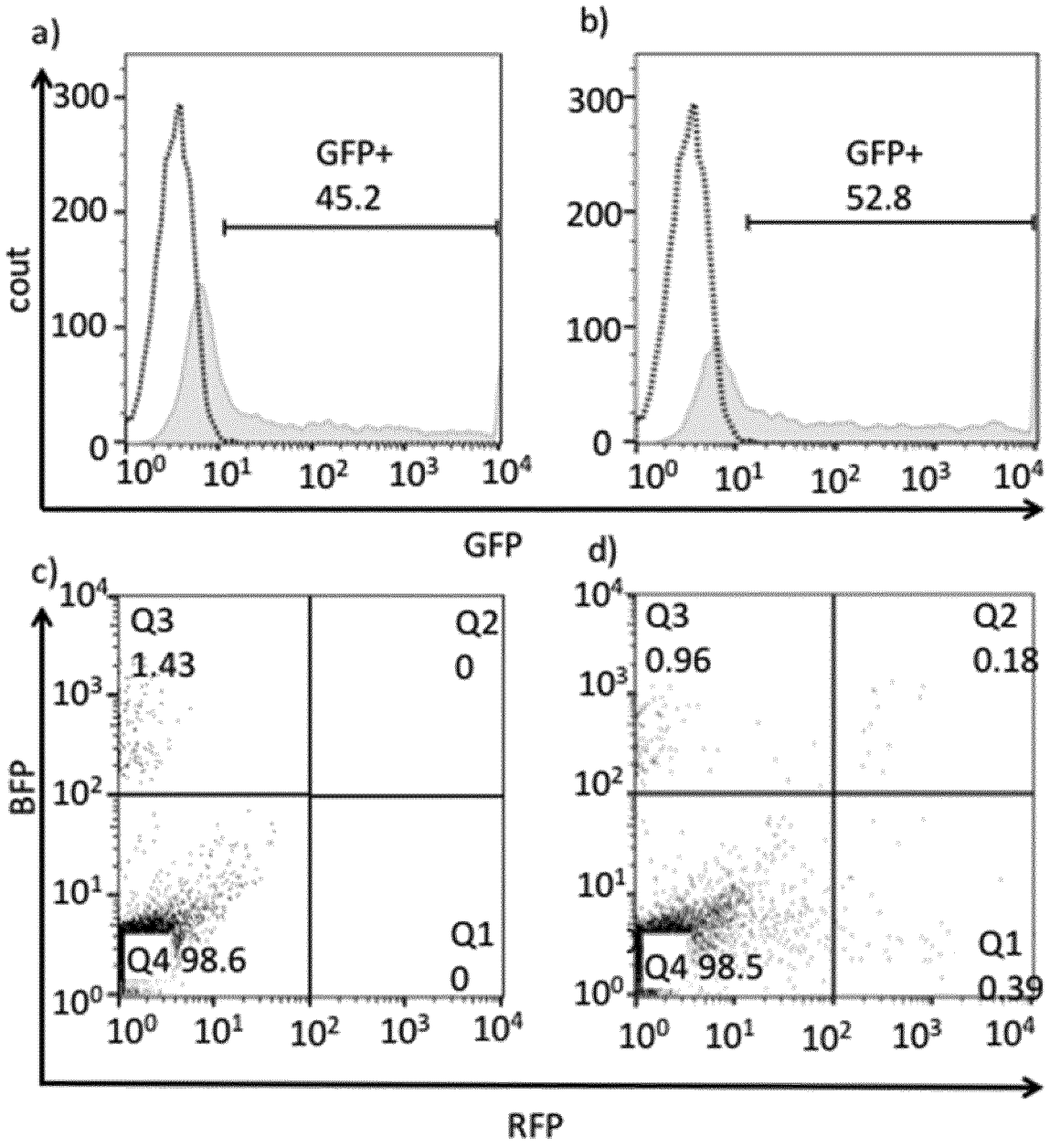

a)
b)
300
200
100
0
cout
GFP+
45.2
GFP+
52.8
10^0 10^1 10^2 10^3 10^4
GFP
c)
d)
10^4
10^3
10^2
10^1
10^0
BFP
Q3
1.43
Q2
0
Q4 98.6
Q1
0
Q3
0.96
Q2
0.18
Q4 98.5
Q1
0.39
RFP

Figure 32

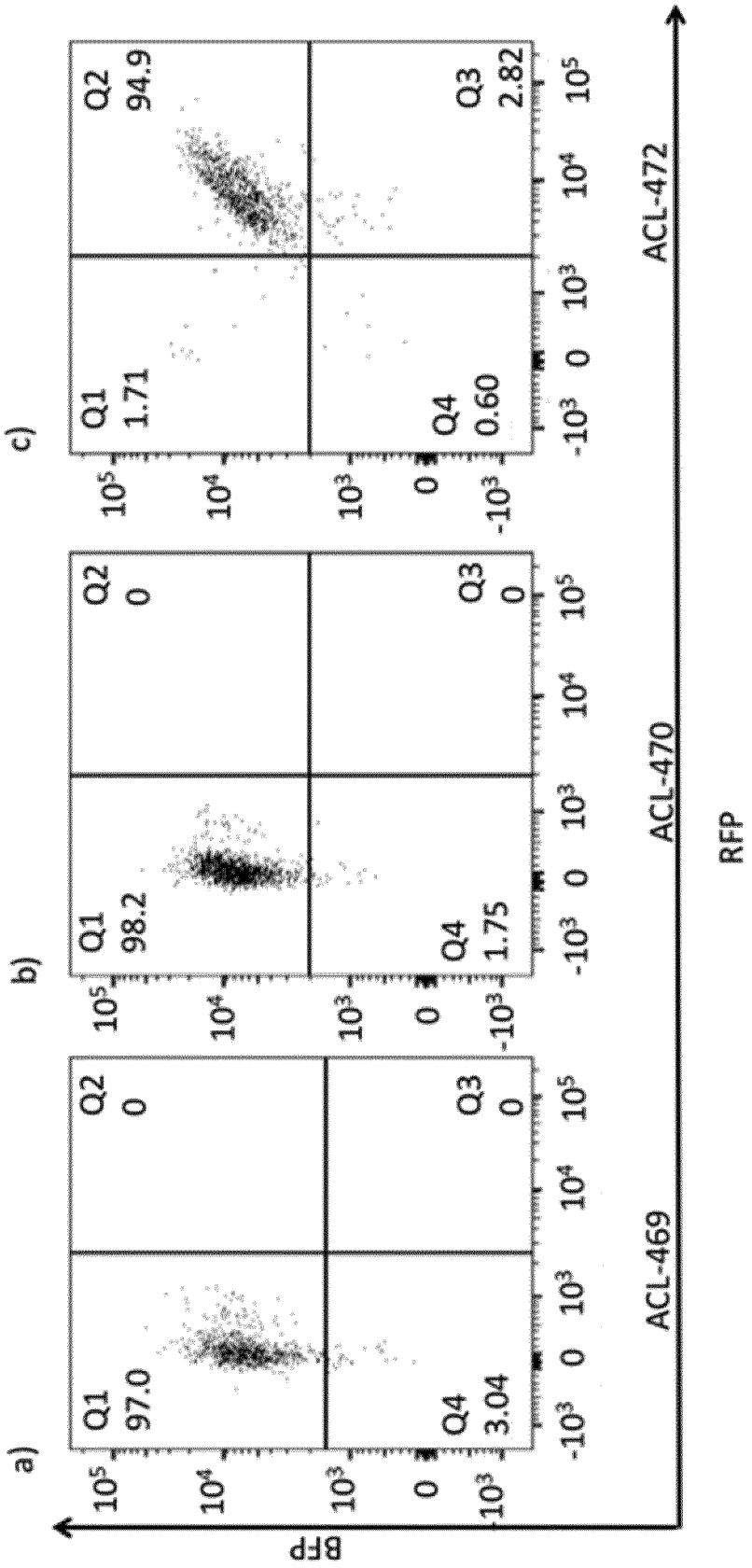

a)
b)
c)
Q1
97.0
Q2
0
Q3
0
Q4
3.04
Q1
98.2
Q2
0
Q3
0
Q4
1.75
Q1
1.71
Q2
94.9
Q3
2.82
Q4
0.60
BFP
ACL-469
ACL-470
ACL-472
RFP

Figure 33

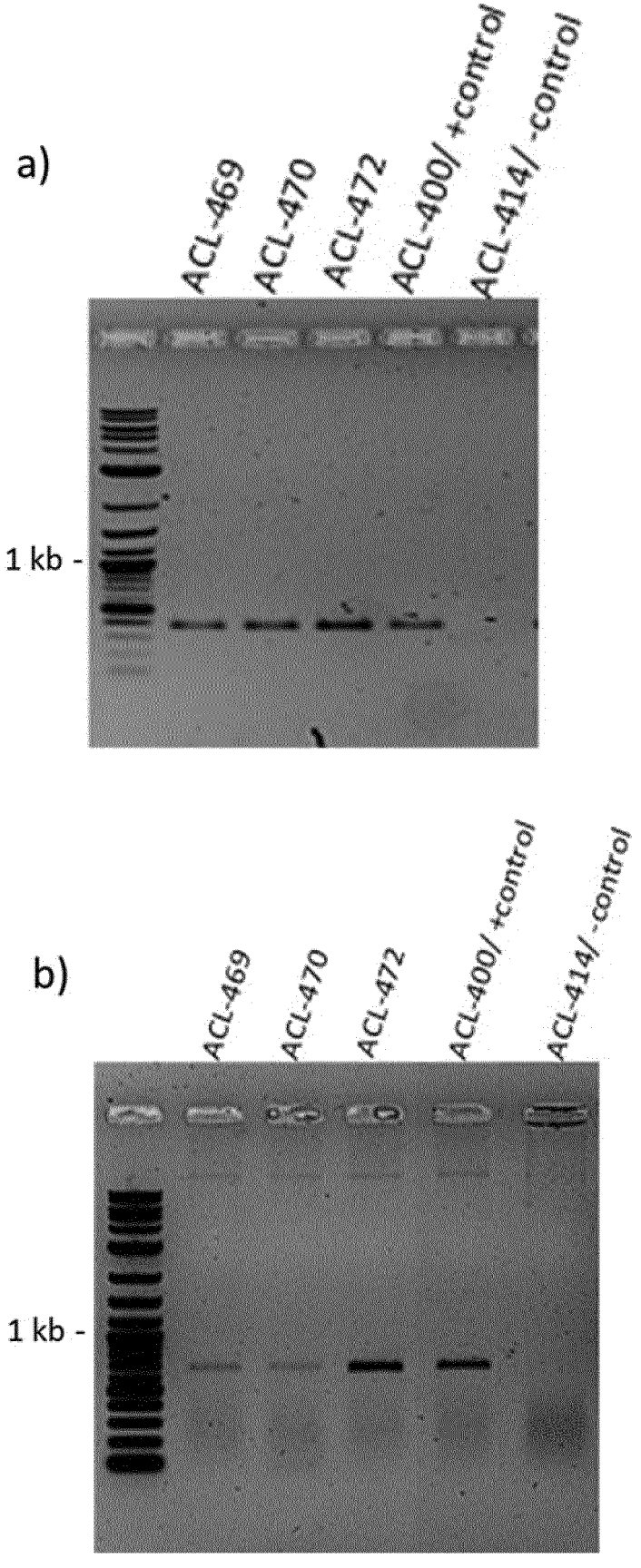

a)
ACL-469
ACL-470
ACL-472
ACL-400/ +control
ACL-414/ -control
1 kb -
b)
ACL-469
ACL-470
ACL-472
ACL-400/ +control
ACL-414/ -control
1 kb -

Figure 34

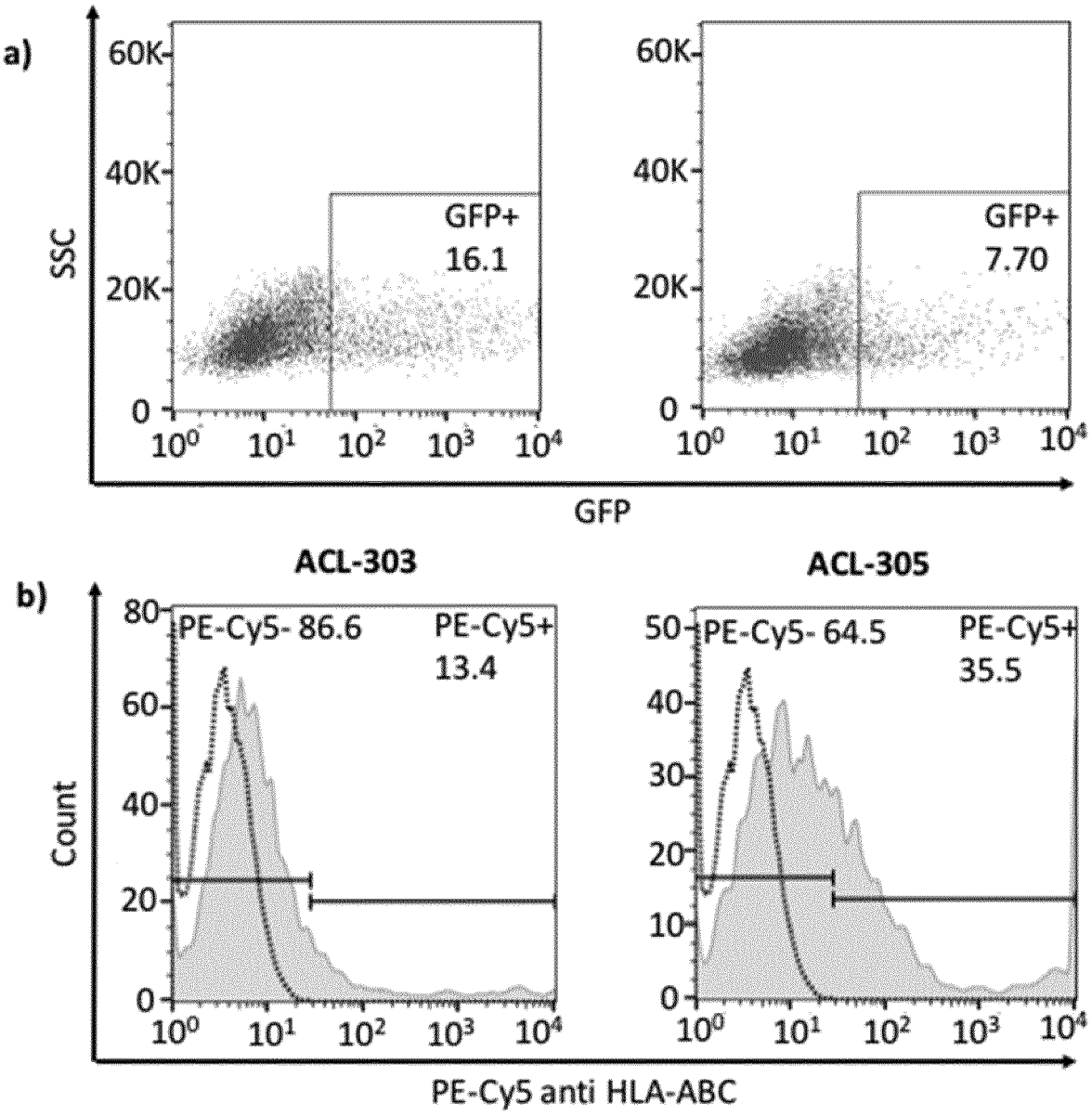

a)
60K
40K
20K
0
SSC
GFP+
16.1
GFP+
7.70
10^0 10^1 10^2 10^3 10^4
GFP
b)
ACL-303
ACL-305
80
60
40
20
0
Count
PE-Cy5- 86.6
PE-Cy5+
13.4
50
40
30
20
10
PE-Cy5- 64.5
PE-Cy5+
35.5
PE-Cy5 anti HLA-ABC

Figure 35

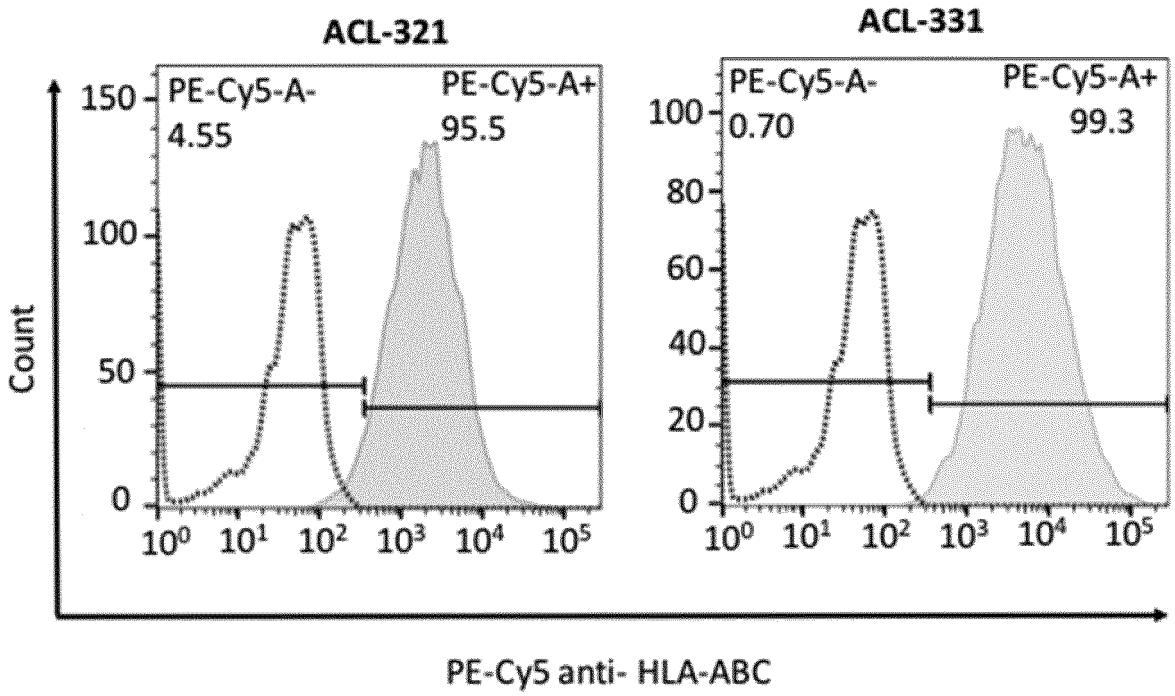

ACL-321
PE-Cy5-A-
4.55
PE-Cy5-A+
95.5
ACL-331
PE-Cy5-A-
0.70
PE-Cy5-A+
99.3
Count
PE-Cy5 anti- HLA-ABC

Figure 36

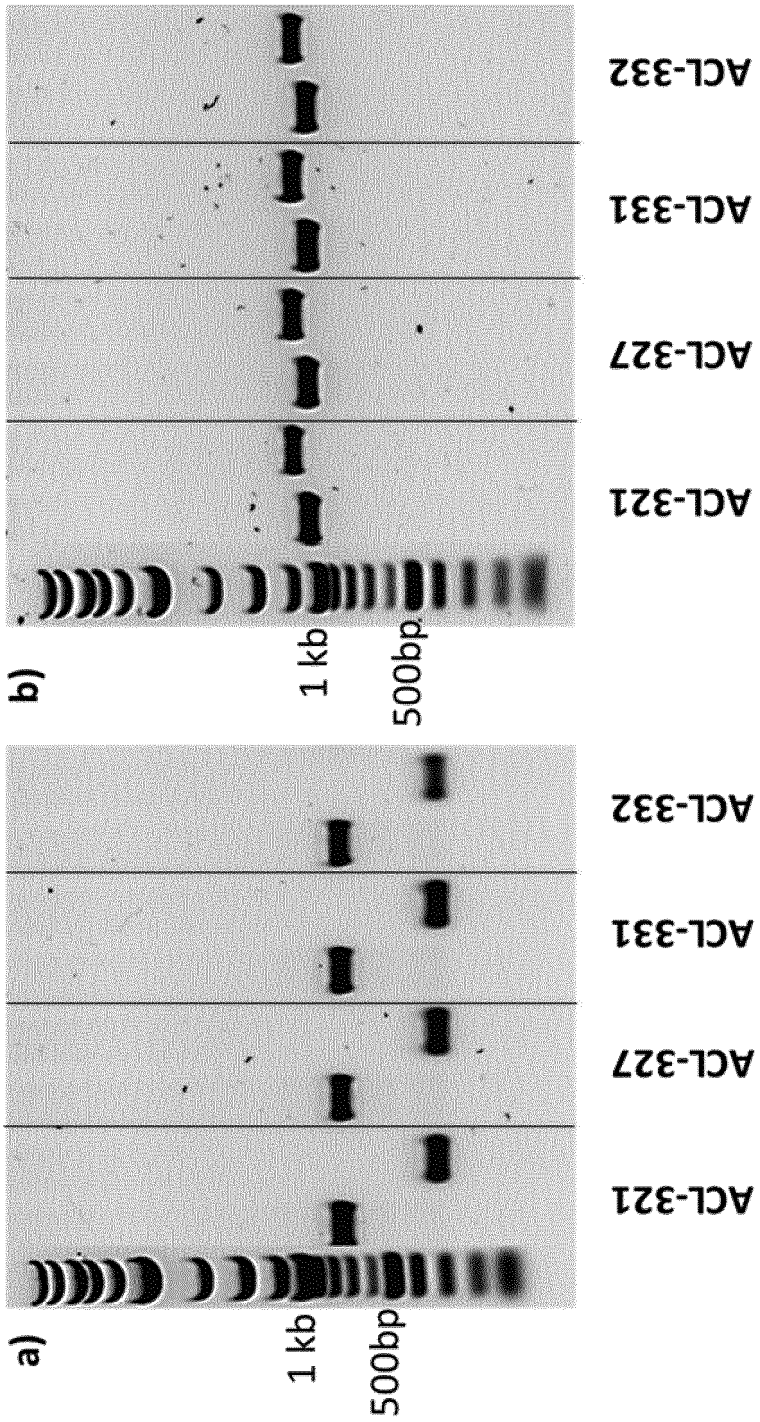

ACL-332
ACL-331
ACL-327
ACL-321
b)
1 kb
500bp
ACL-332
ACL-331
ACL-327
ACL-321
a)
1 kb
500bp

Figure 37

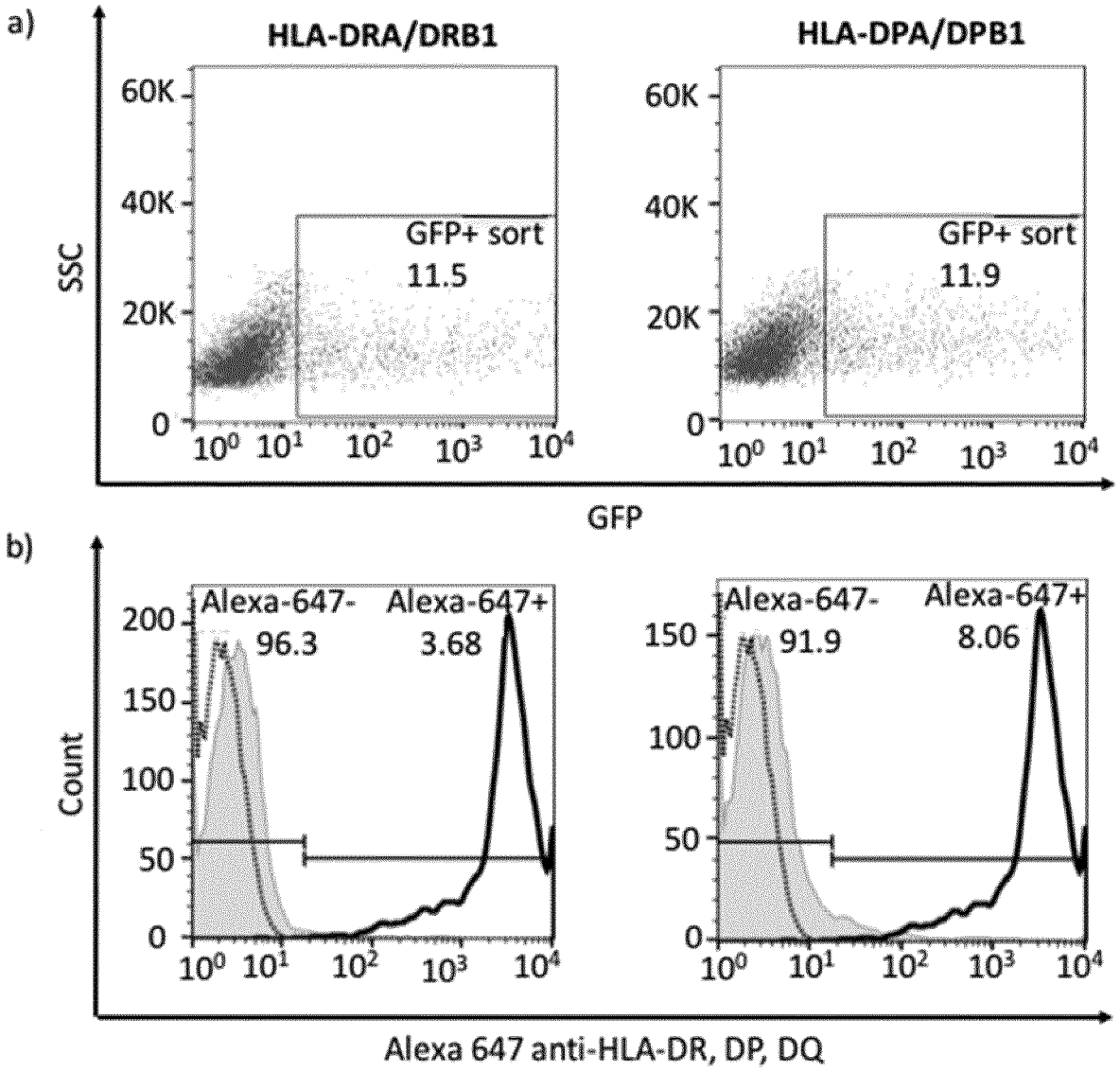

a)
HLA-DRA/DRB1
HLA-DPA/DPB1
60K
40K
20K
0
SSC
GFP+ sort
11.5
GFP+ sort
11.9
10^0 10^1 10^2 10^3 10^4
GFP
b)
Alexa-647-
96.3
Alexa-647+
3.68
Alexa-647-
91.9
Alexa-647+
8.06
200
150
100
50
0
Count
Alexa 647 anti-HLA-DR, DP, DQ

Figure 38

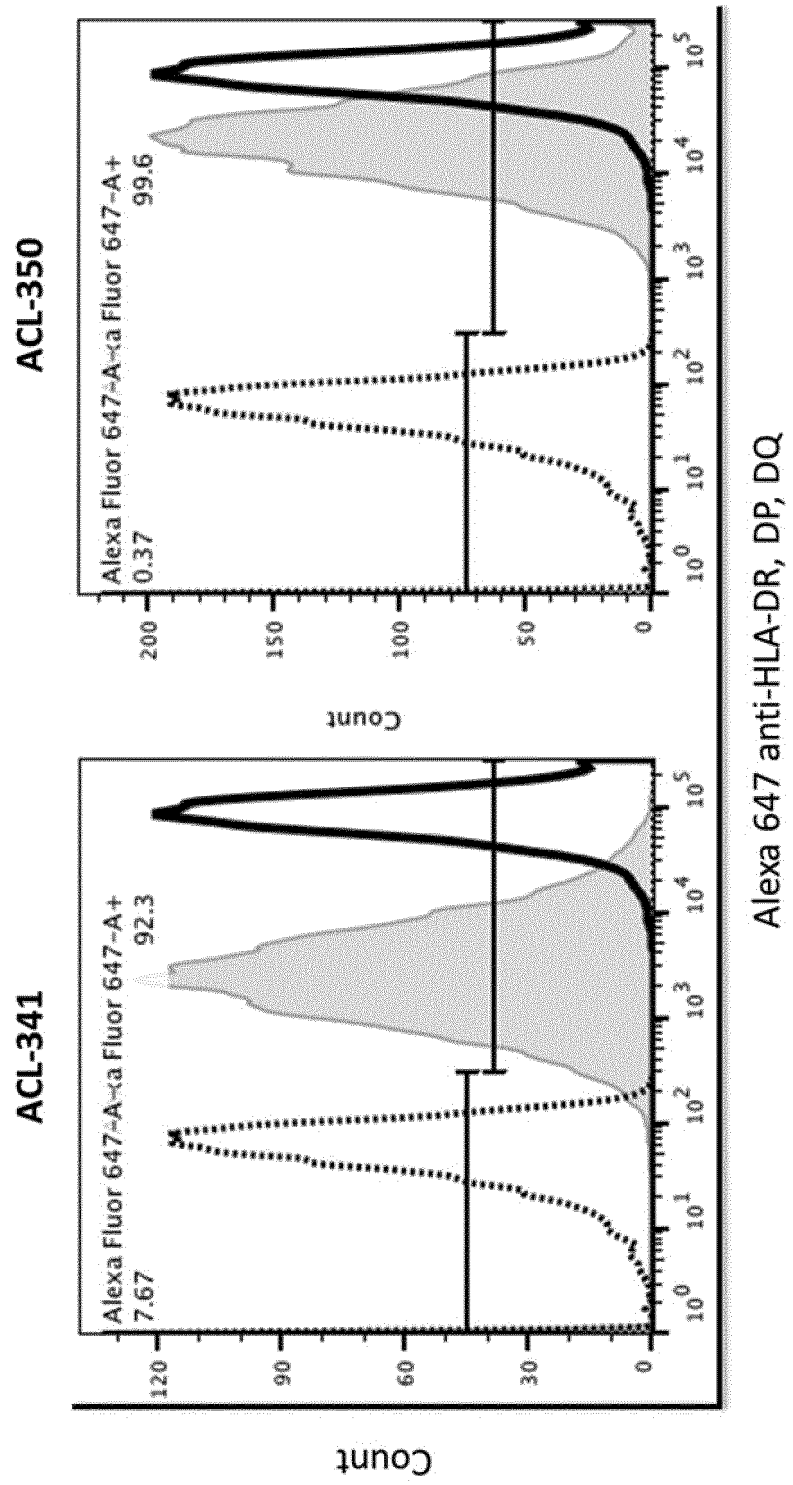

ACL-350
ACL-341
Alexa Fluor 647-A
Alexa Fluor 647-A+
0.37
99.6
7.67
92.3
Count
Alexa 647 anti-HLA-DR, DP, DQ

Figure 39

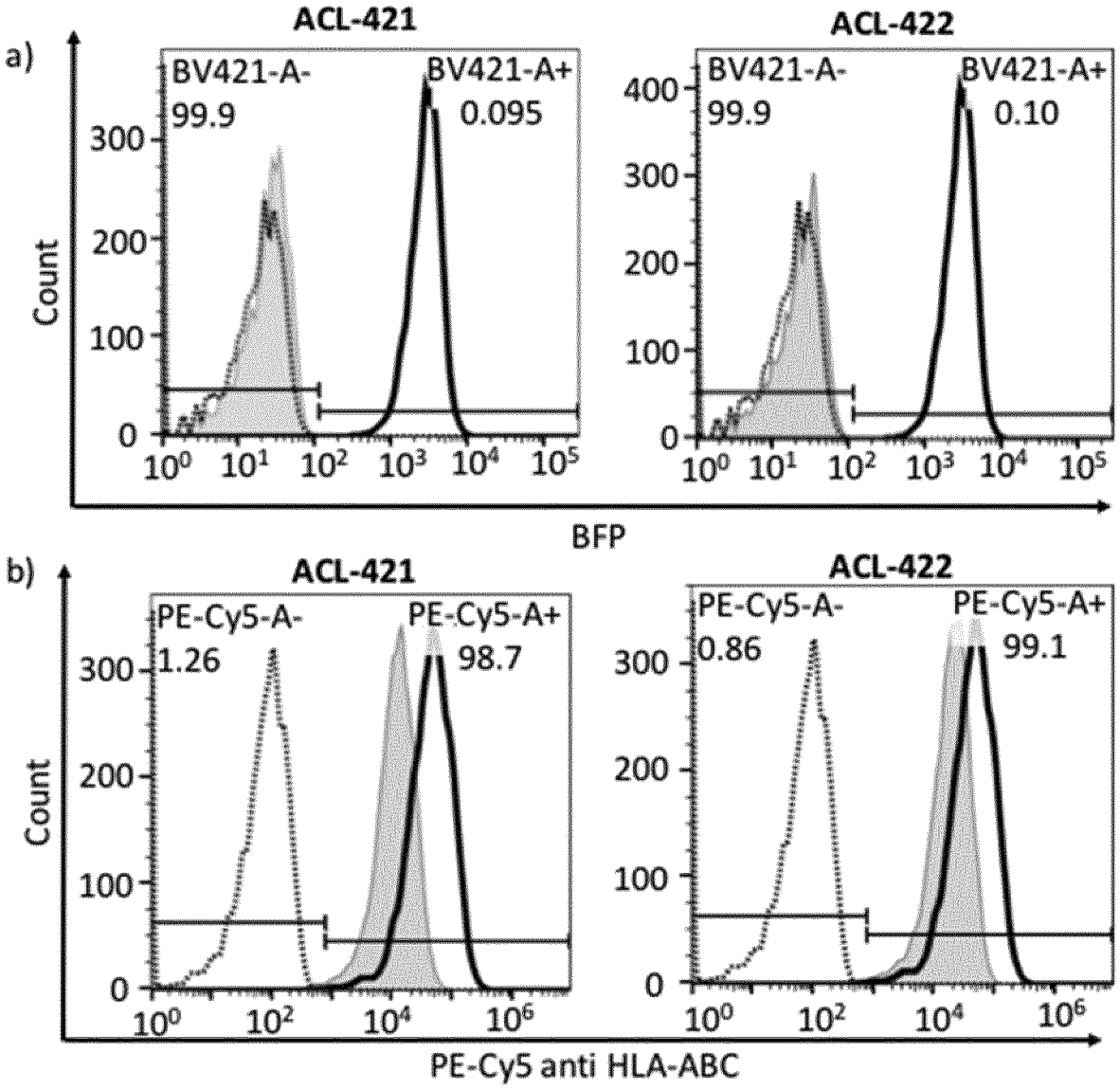

a)
ACL-421
BV421-A-
99.9
BV421-A+
0.095
ACL-422
BV421-A-
99.9
BV421-A+
0.10
Count
BFP
b)
ACL-421
PE-Cy5-A-
1.26
PE-Cy5-A+
98.7
ACL-422
PE-Cy5-A-
0.86
PE-Cy5-A+
99.1
Count
PE-Cy5 anti HLA-ABC

Figure 40

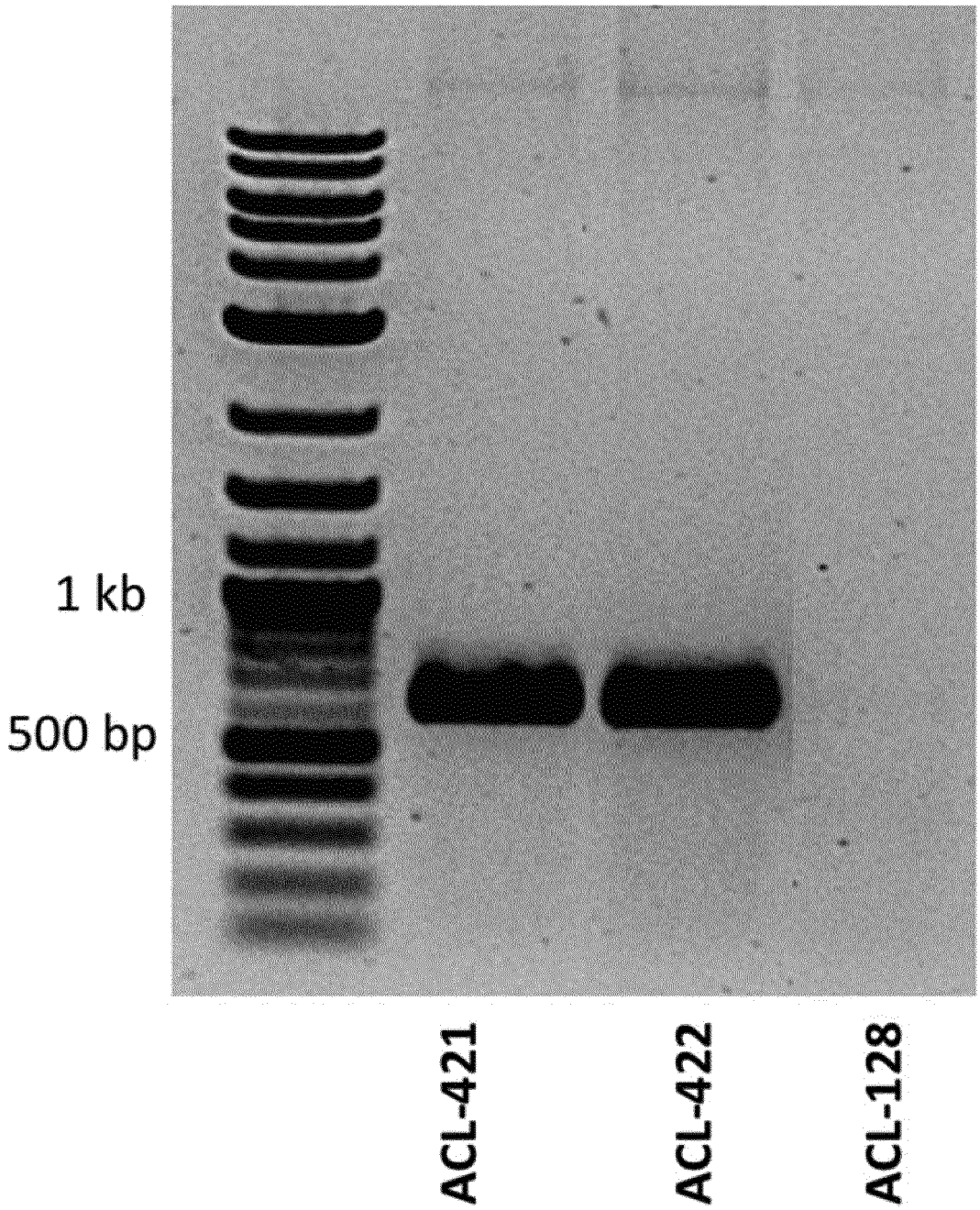


Figure 41

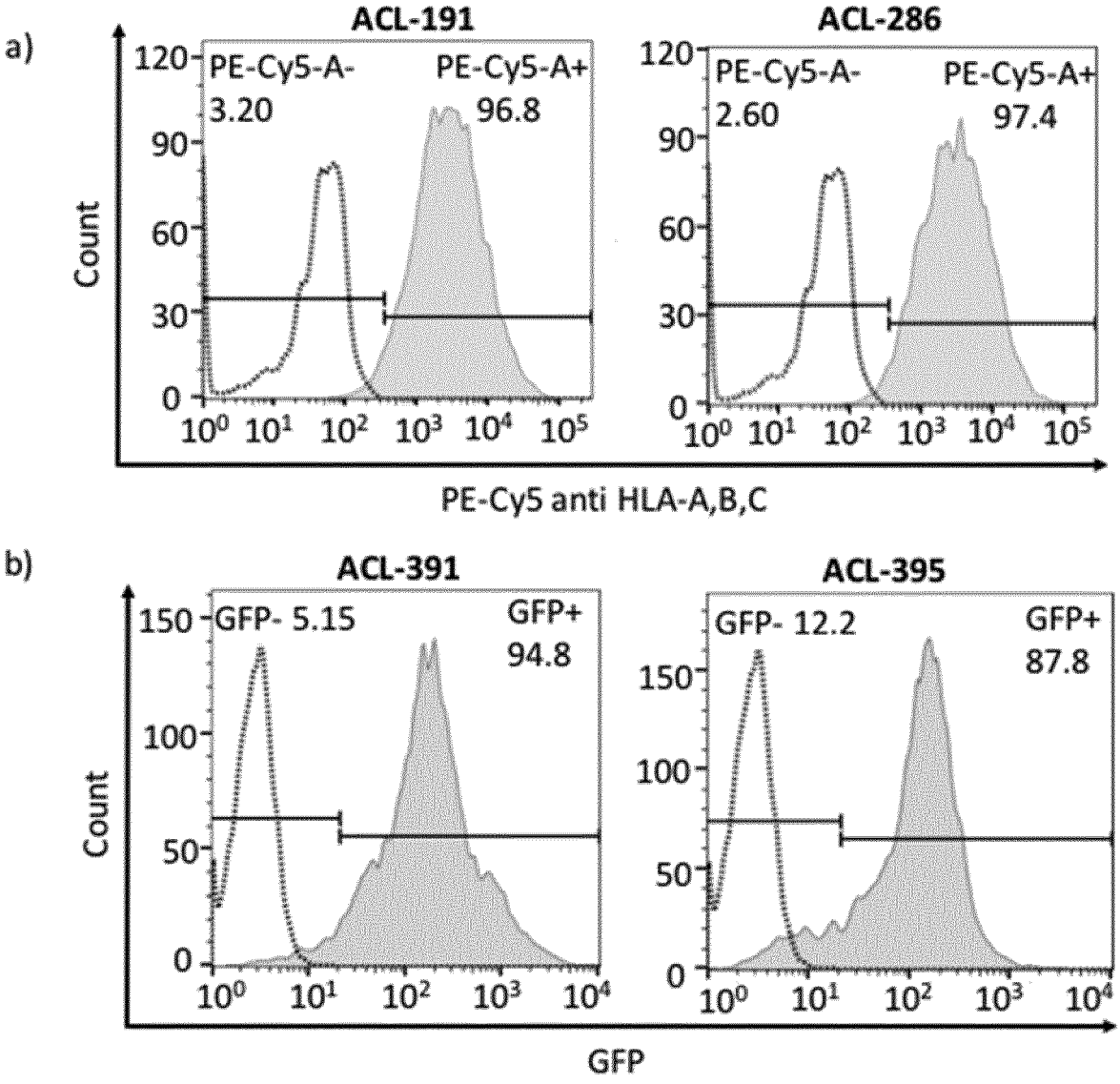

a)
ACL-191
ACL-286
PE-Cy5-A-
3.20
PE-Cy5-A+
96.8
PE-Cy5-A-
2.60
PE-Cy5-A+
97.4
Count
PE-Cy5 anti HLA-A,B,C
b)
ACL-391
ACL-395
GFP- 5.15
GFP+
94.8
GFP- 12.2
GFP+
87.8
Count
GFP

Figure 42

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 43

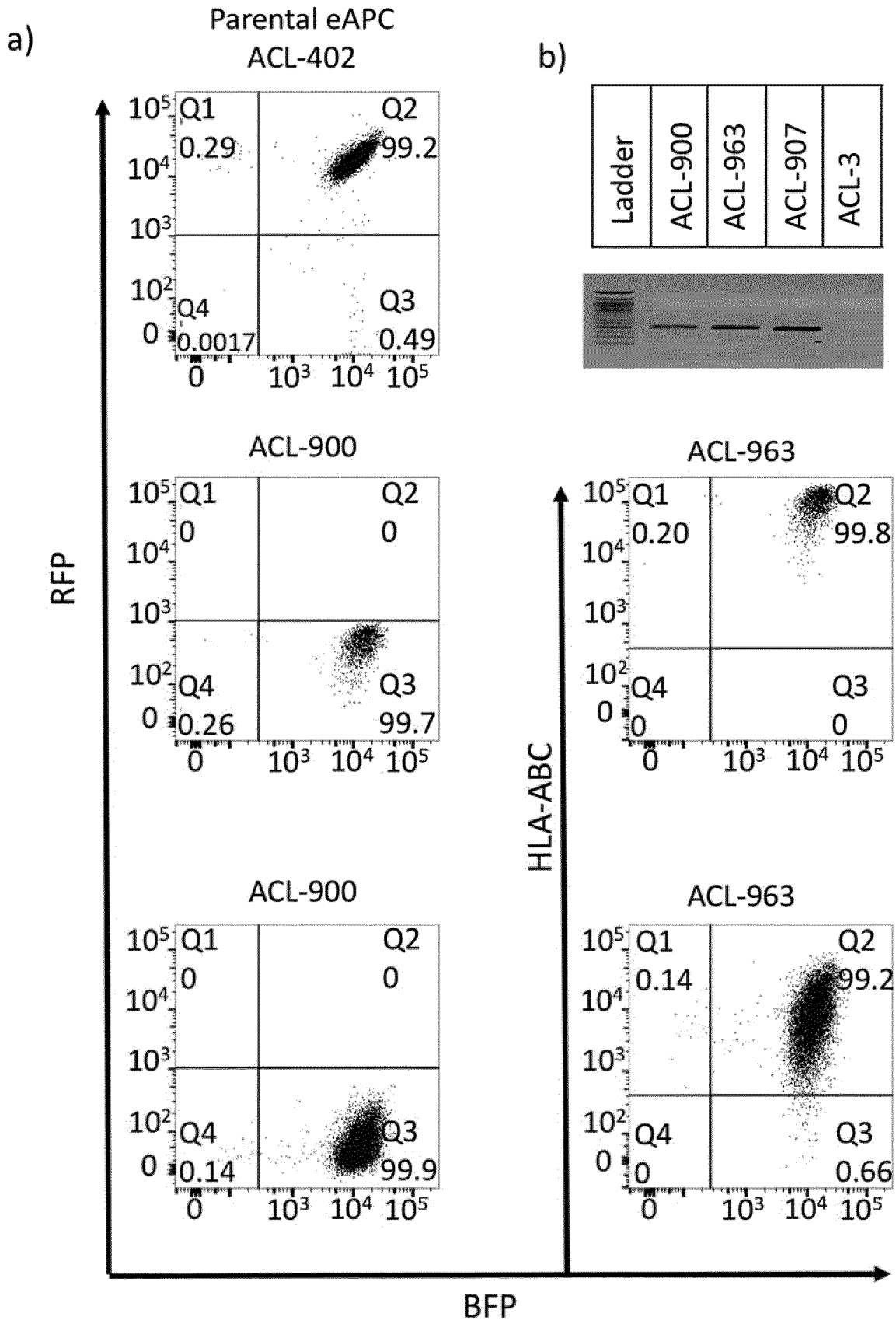

a)
Parental eAPC
ACL-402
Q1
0.29
Q2
99.2
Q4
0.0017
Q3
0.49
ACL-900
Q1
0
Q2
0
Q4
0.26
Q3
99.7
ACL-900
Q1
0
Q2
0
Q4
0.14
Q3
99.9
b)
Ladder
ACL-900
ACL-963
ACL-907
ACL-3
ACL-963
Q1
0.20
Q2
99.8
Q4
0
Q3
0
ACL-963
Q1
0.14
Q2
99.2
Q4
0
Q3
0.66
RFP
HLA-ABC
BFP
0
10^3
10^4
10^5
10^2

Figure 44

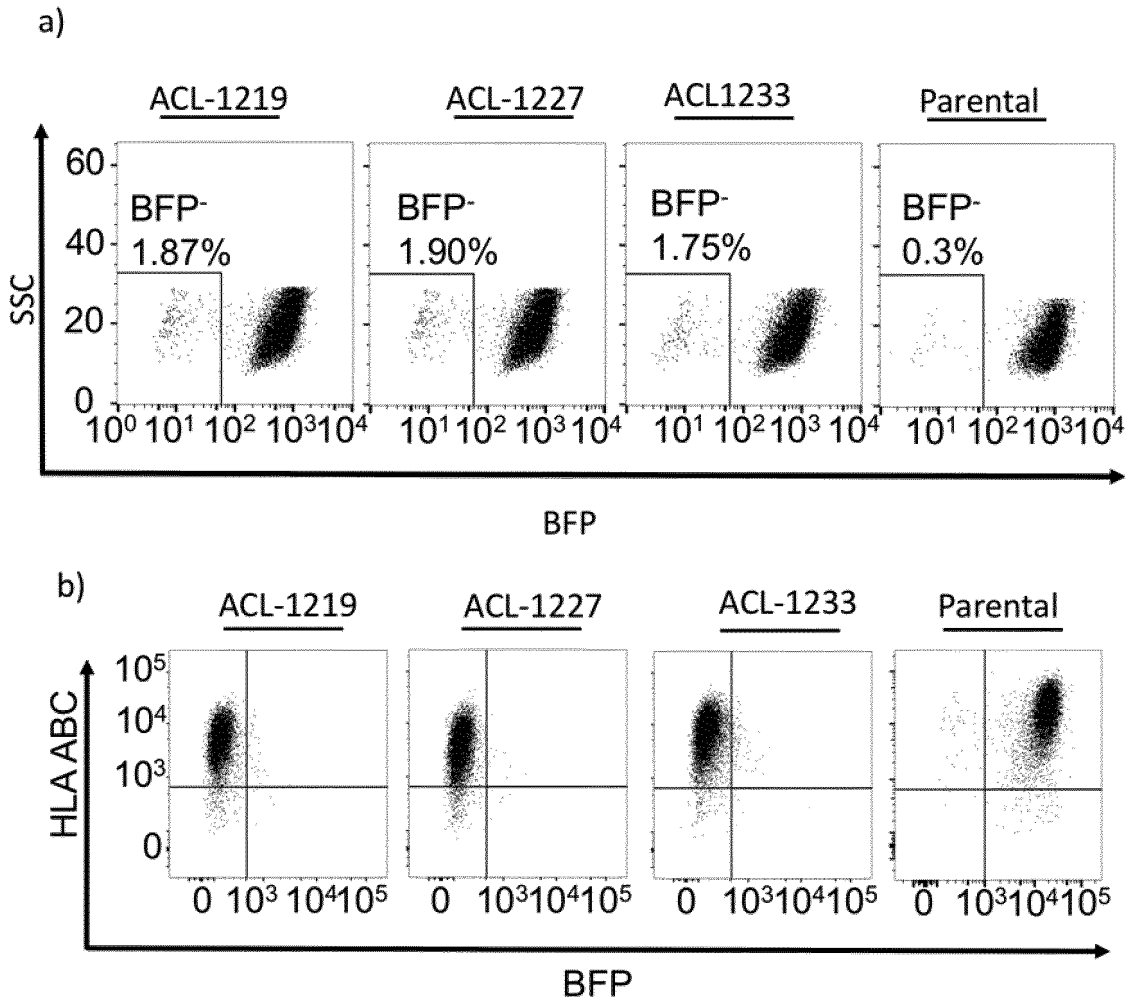

a)
ACL-1219
ACL-1227
ACL1233
Parental
BFP-
1.87%
BFP-
1.90%
BFP-
1.75%
BFP-
0.3%
SSC
BFP
b)
ACL-1219
ACL-1227
ACL-1233
Parental
HLA ABC
BFP
c)
1 2 3 4 5 6 7 8 9 10
10kb-
3.0kb-
2.0kb-
1.5kb-
1.0kb-
0.8kb-
0.5kb-
0.1kb-
pp28HCMV ORF
pp52HCMV ORF
pp65HCMV ORF

Figure 45

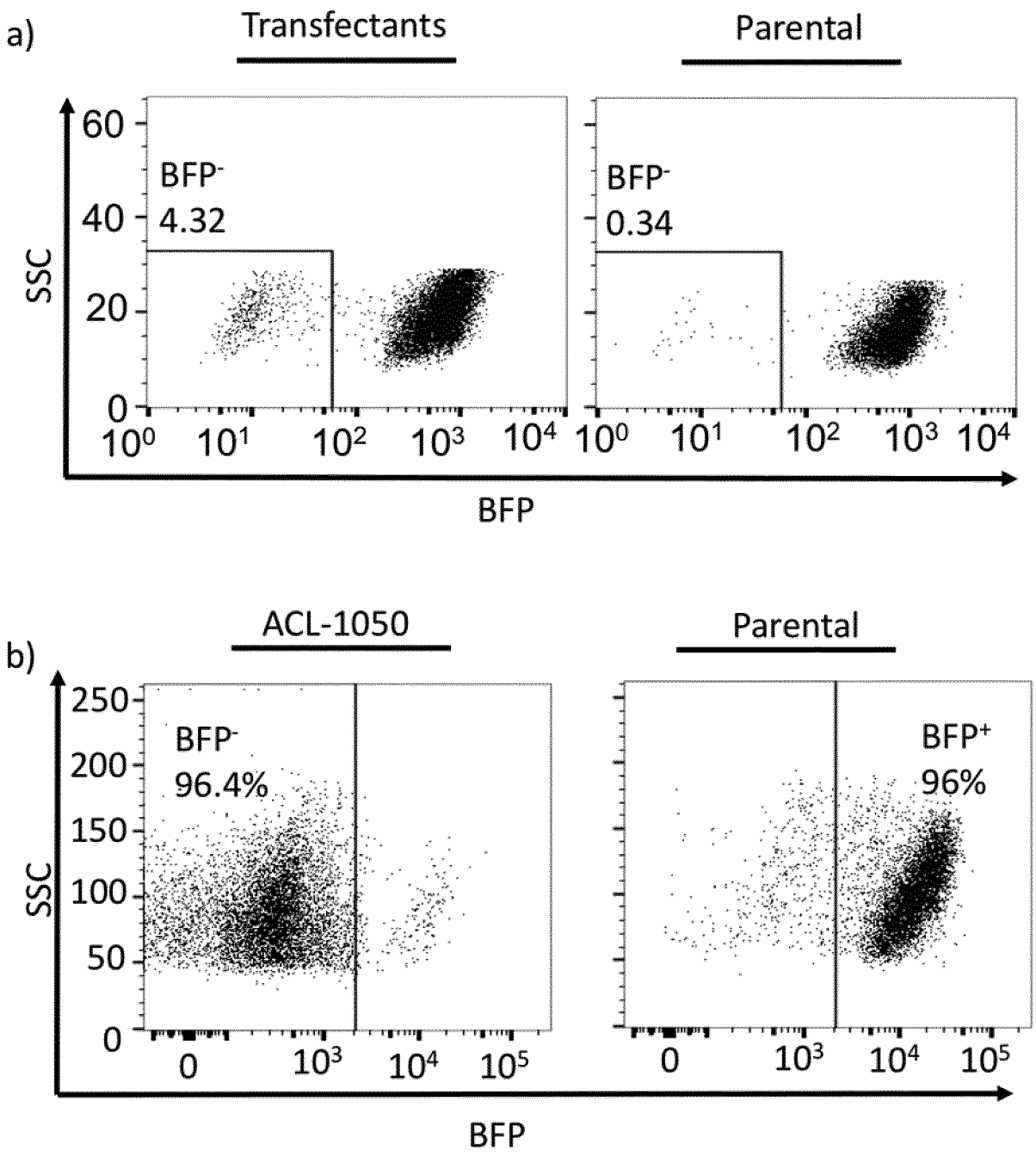

a)
Transfectants
Parental
BFP-
4.32
BFP-
0.34
SSC
BFP
b)
ACL-1050
Parental
BFP-
96.4%
BFP+
96%
SSC
BFP

Figure 45

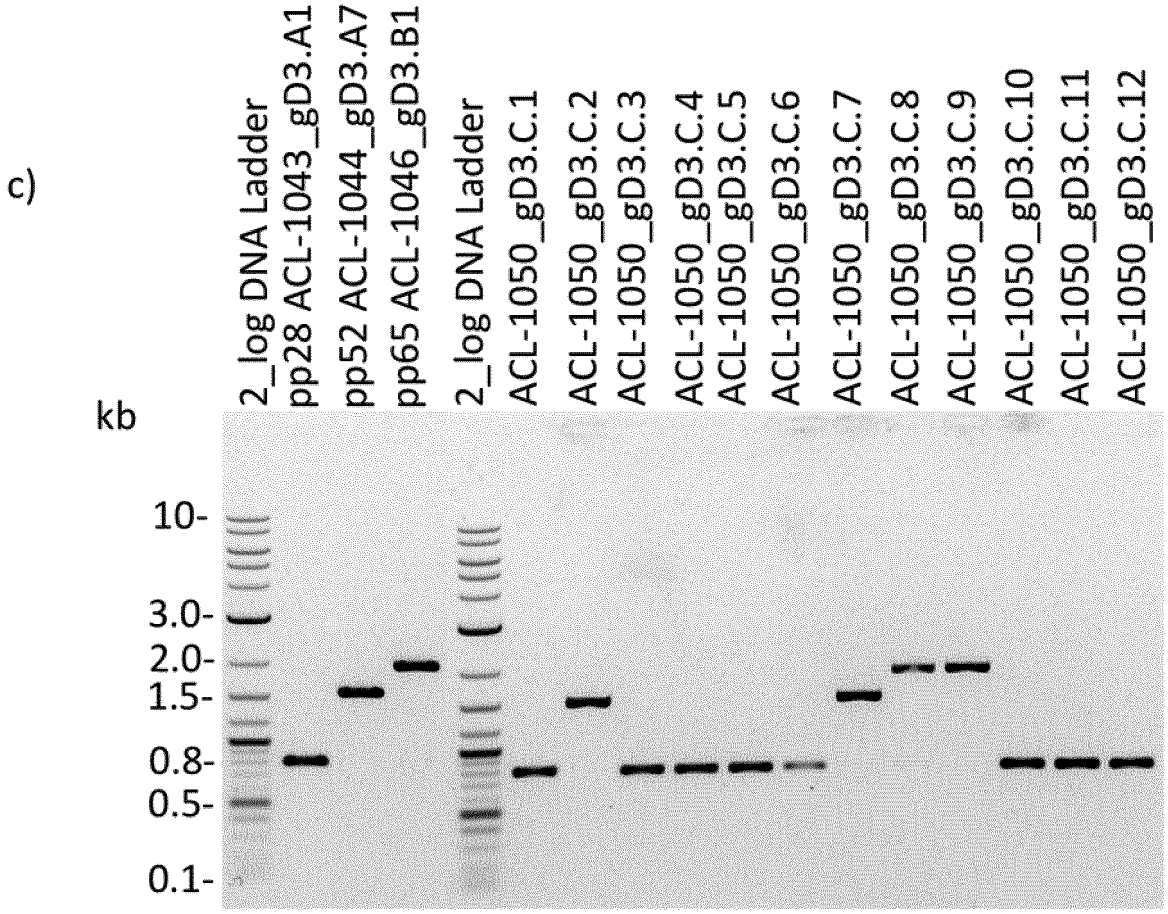

c)
kb
10-
3.0-
2.0-
1.5-
0.8-
0.5-
0.1-
2_log DNA Ladder
pp28 ACL-1043_gD3.A1
pp52 ACL-1044_gD3.A7
pp65 ACL-1046_gD3.B1
2_log DNA Ladder
ACL-1050_gD3.C.1
ACL-1050_gD3.C.2
ACL-1050_gD3.C.3
ACL-1050_gD3.C.4
ACL-1050_gD3.C.5
ACL-1050_gD3.C.6
ACL-1050_gD3.C.7
ACL-1050_gD3.C.8
ACL-1050_gD3.C.9
ACL-1050_gD3.C.10
ACL-1050_gD3.C.11
ACL-1050_gD3.C.12

Figure 46

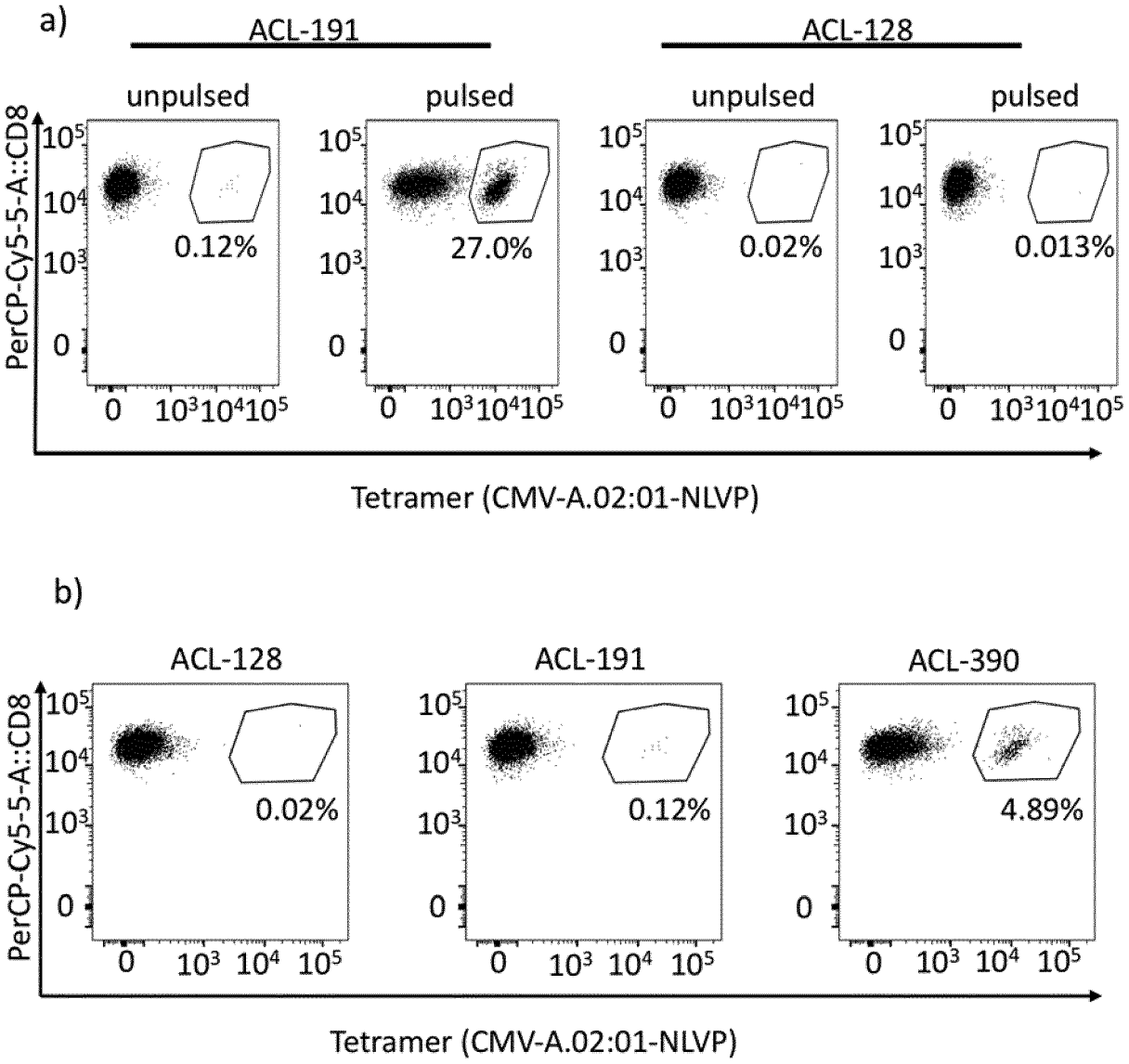

a)
ACL-191
ACL-128
unpulsed
pulsed
unpulsed
pulsed
PerCP-Cy5-5-A::CD8
0.12%
27.0%
0.02%
0.013%
Tetramer (CMV-A.02:01-NLVP)
b)
ACL-128
ACL-191
ACL-390
PerCP-Cy5-5-A::CD8
0.02%
0.12%
4.89%
Tetramer (CMV-A.02:01-NLVP)

Figure 47

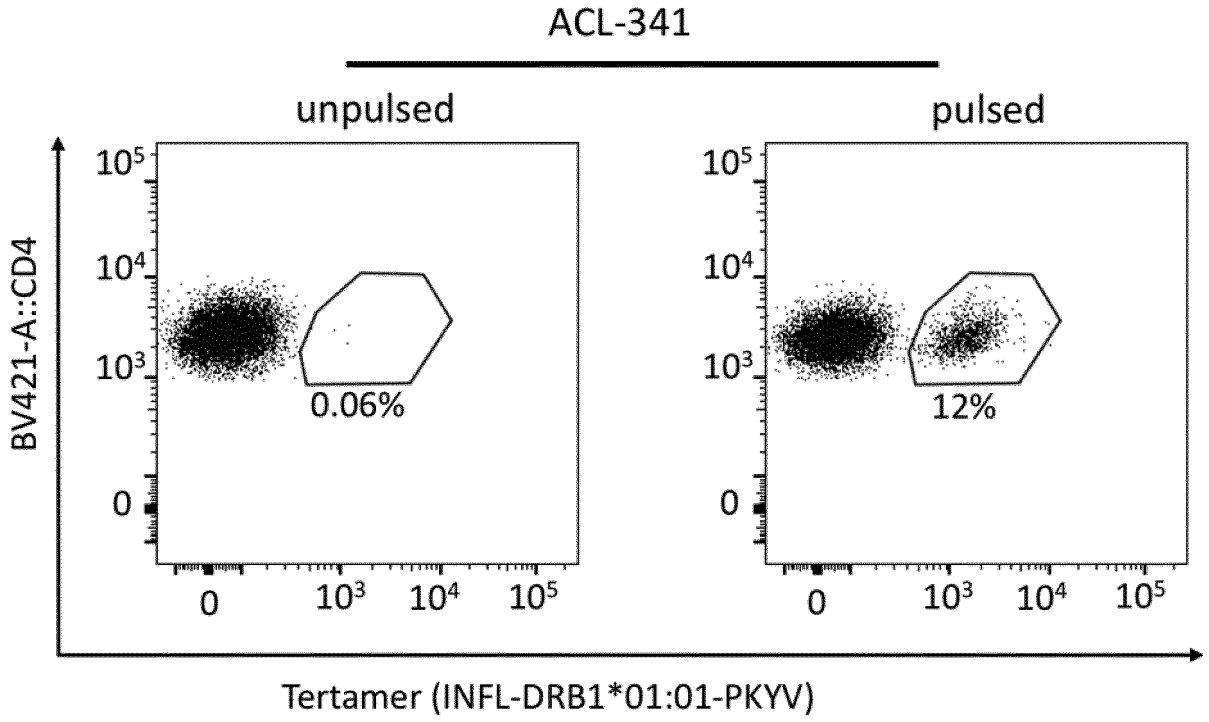

ACL-341
unpulsed
pulsed
BV421-A::CD4
10^5
10^4
10^3
0
0.06%
12%
0
10^3
10^4
10^5
Tertamer (INFL-DRB1*01:01-PKYV)

Figure 48

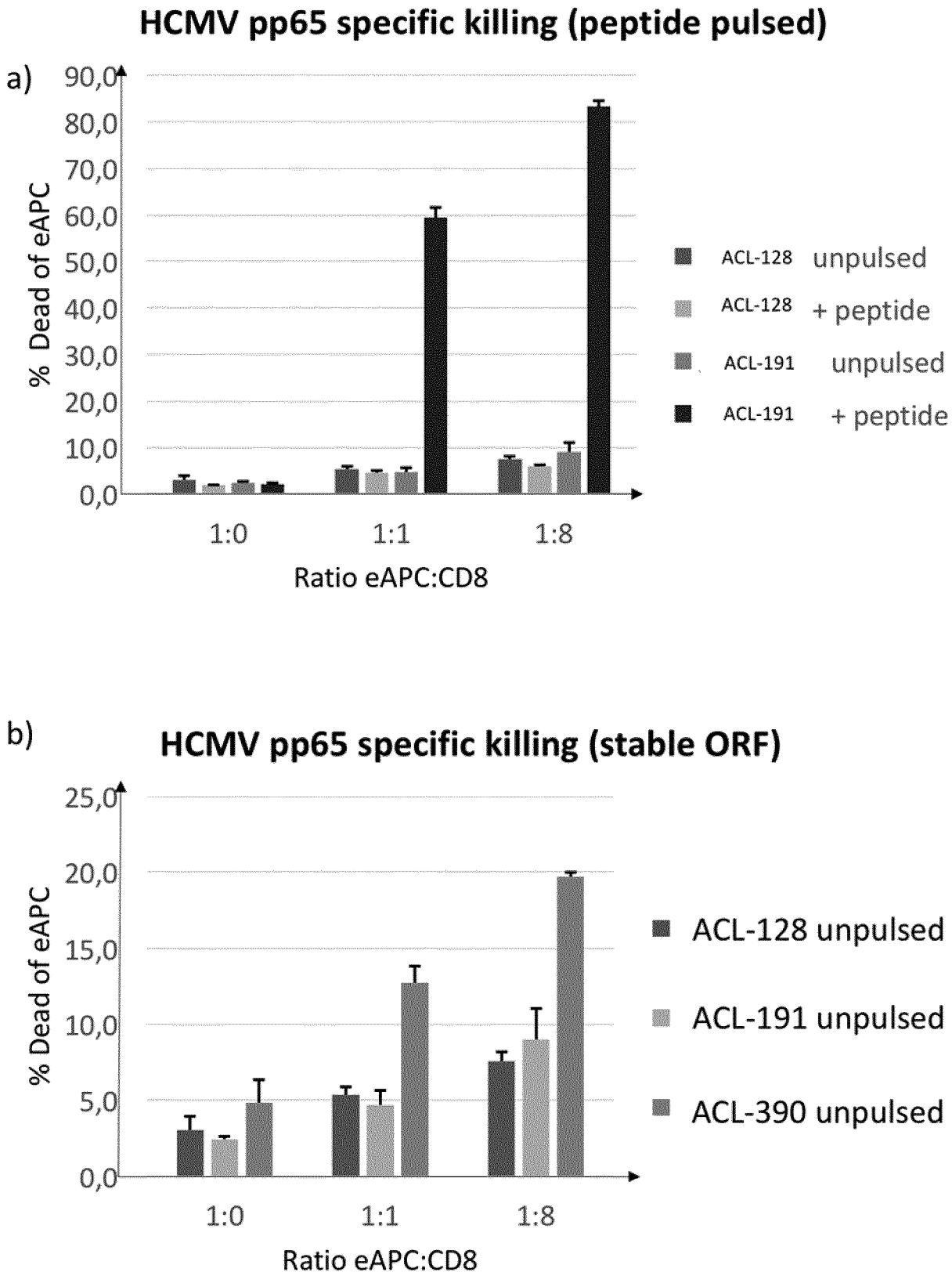

HCMV pp65 specific killing (peptide pulsed)
a)
% Dead of eAPC
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
1:0
1:1
1:8
Ratio eAPC:CD8
ACL-128 unpulsed
ACL-128 + peptide
ACL-191 unpulsed
ACL-191 + peptide
b)
HCMV pp65 specific killing (stable ORF)
% Dead of eAPC
25,0
20,0
15,0
10,0
5,0
0,0
1:0
1:1
1:8
Ratio eAPC:CD8
ACL-128 unpulsed
ACL-191 unpulsed
ACL-390 unpulsed

Figure 49

ID	eAPC line ID	eAPC allele	Peptide sequence	Identified peptides	Intensity	PEP score
1	ACL-900	HLA-A*02:01	NA	NA	0	0
2	ACL-900	HLA-A*02:01	NLVPMVATV	NLVPMVATV	3.4E+07	6.55E-07
4	ACL-900	HLA-A*02:01	NLGPMAAGV	NA	0	0
10	ACL-963	HLA-A*24:02	NA	NA	0	0
11	ACL-963	HLA-A*24:02	VYALPLKML	VYALPLKML	7.9E+06	0.026368
12	ACL-963	HLA-A*24:02	NLVPMVATV	NA	0	0
13	ACL-900	HLA-A*02:01	VYALPLKML	NA	0	0