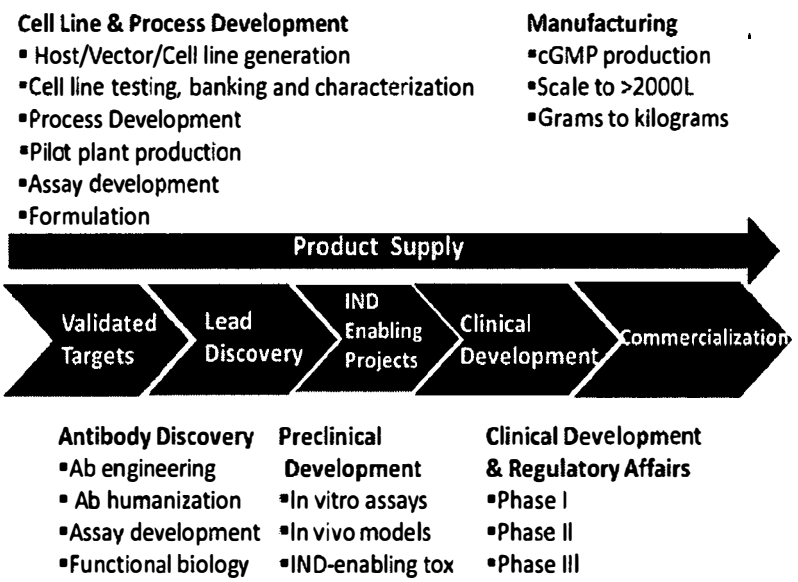
A.


Cell Line & Process Development
▪ Host/Vector/Cell line generation
▪Cell line testing, banking and characterization
▪Process Development
▪Pilot plant production
▪Assay development
▪Formulation
Manufacturing
▪cGMP production
▪Scale to >2000L
▪Grams to kilograms
Product Supply
Validated Targets
Lead Discovery
IND Enabling Projects
Clinical Development
Commercialization
Antibody Discovery
▪Ab engineering
▪ Ab humanization
▪Assay development
▪Functional biology
Preclinical Development
▪In vitro assays
▪In vivo models
▪IND-enabling tox
Clinical Development & Regulatory Affairs
▪Phase I
▪Phase II
▪Phase III

B.

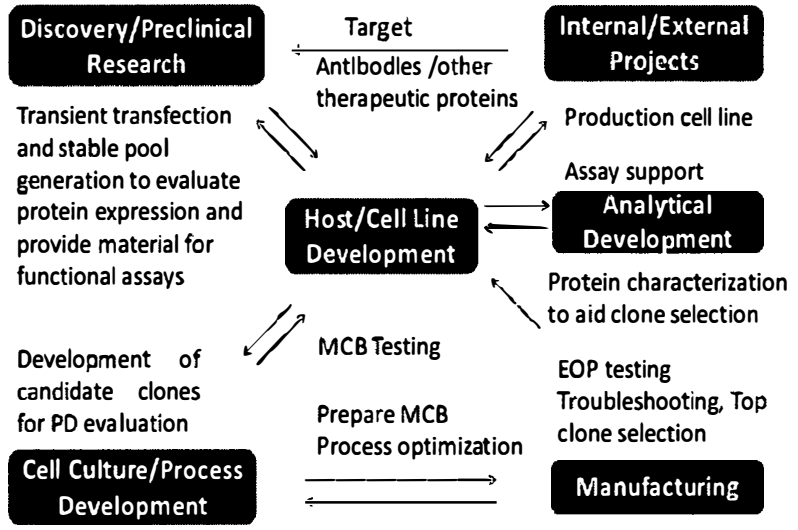

Discovery/Preclinical Research
Target
Antibodies /other therapeutic proteins
Internal/External Projects
Transient transfection and stable pool generation to evaluate protein expression and provide material for functional assays
Production cell line
Assay support
Host/Cell Line Development
Analytical Development
Protein characterization to aid clone selection
MCB Testing
Development of candidate clones for PD evaluation
EOP testing
Troubleshooting, Top clone selection
Prepare MCB
Process optimization
Cell Culture/Process Development
Manufacturing

FIG. 1

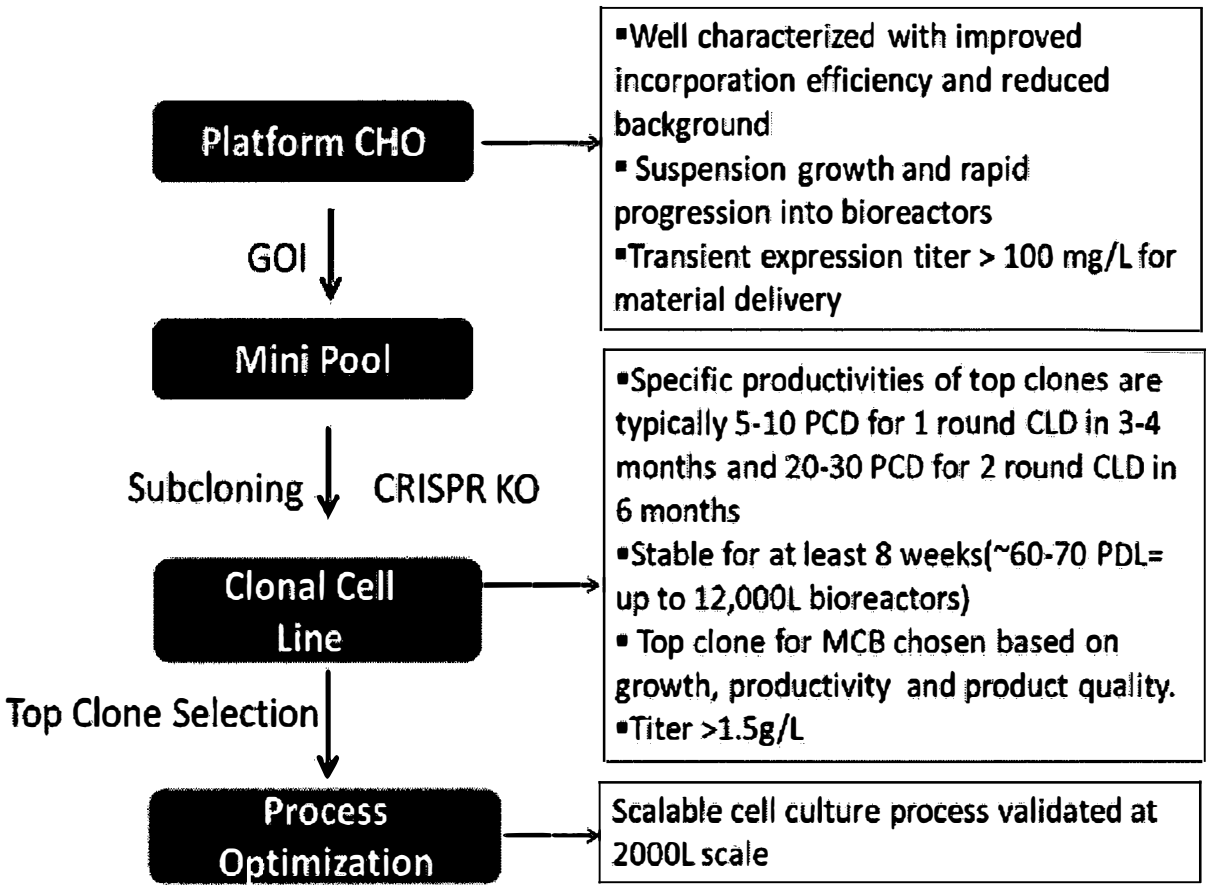

Platform CHO
GOI
Mini Pool
Subcloning
CRISPR KO
Clonal Cell Line
Top Clone Selection
Process Optimization
▪Well characterized with improved incorporation efficiency and reduced background
▪ Suspension growth and rapid progression into bioreactors
▪Transient expression titer > 100 mg/L for material delivery
▪Specific productivities of top clones are typically 5-10 PCD for 1 round CLD in 3-4 months and 20-30 PCD for 2 round CLD in 6 months
▪Stable for at least 8 weeks(~60-70 PDL= up to 12,000L bioreactors)
▪ Top clone for MCB chosen based on growth, productivity and product quality.
▪Titer >1.5g/L
Scalable cell culture process validated at 2000L scale

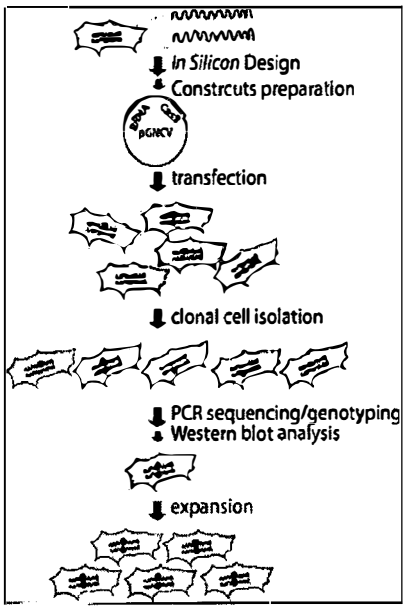
FIG. 2

A.

FACS SCD yields 3 fold of monoclonal outgrowths than traditional LDC

	Seeding Density	Deposition Rate	Outgrowth Rate	Monoclonal Outgrowth Rate
FACS SCD	1 cell/well	63%	53%	49%
LDC	0.5 cell/well	72%	65%	32%

B.

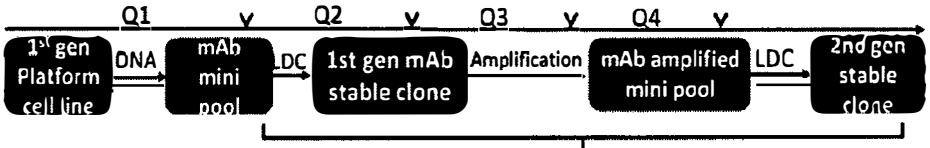


CRISPy program (CHO specific)
- *Zero off-target gRNA sequences*
- *Early exons*

Pooled cells Surveyor assay
- *30-90% KO efficiency*

Screening and Characterization
1. HT targeted DNA sequencing: *50-80% KO efficiency*
2. Western blot analysis *50-90% KO efficiency*
3. Functional assay

C.



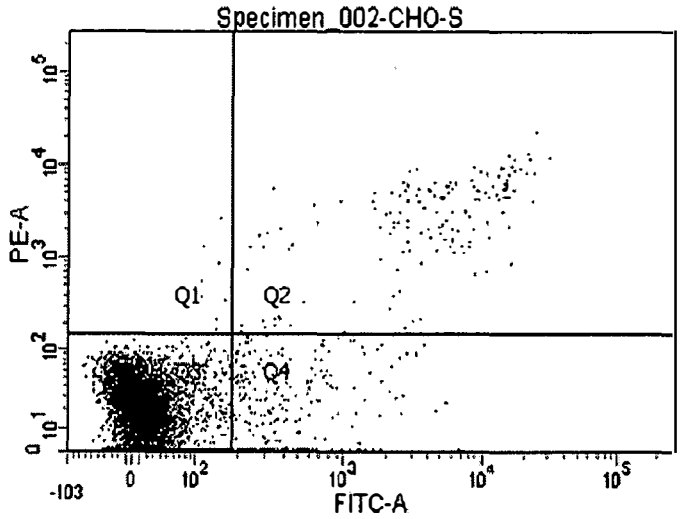
1st gen Platform-GS

Antibody	CLD	Titer g/L	Scale
mAbF-1st	MP-Amp-LDC	1.0	5 L bioreactor
mAbH-1st	MP-LDC-Clonepix	0.5	2000 L bioreactor

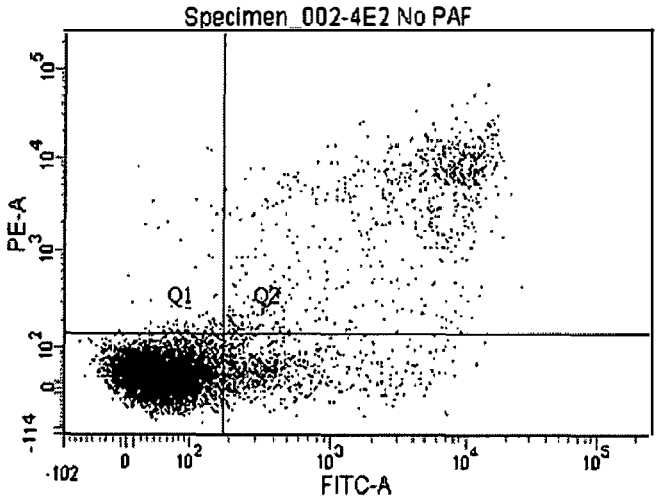
96 Well
24 Well
6 Well
Shake Flask
Bioreactor

FIG. 3

A.



B.



Cell Line	Viable Cell (Q3)	Early Apoptotic Cell (Q4)	Late Apoptotic Cells (Q2)	Dead Cells (Q1)
CHO-S	96 %	2.4 %	1.5 %	0.1 %
4E2	85 %	6.7 %	7.4 %	0.7 %

FIG. 4

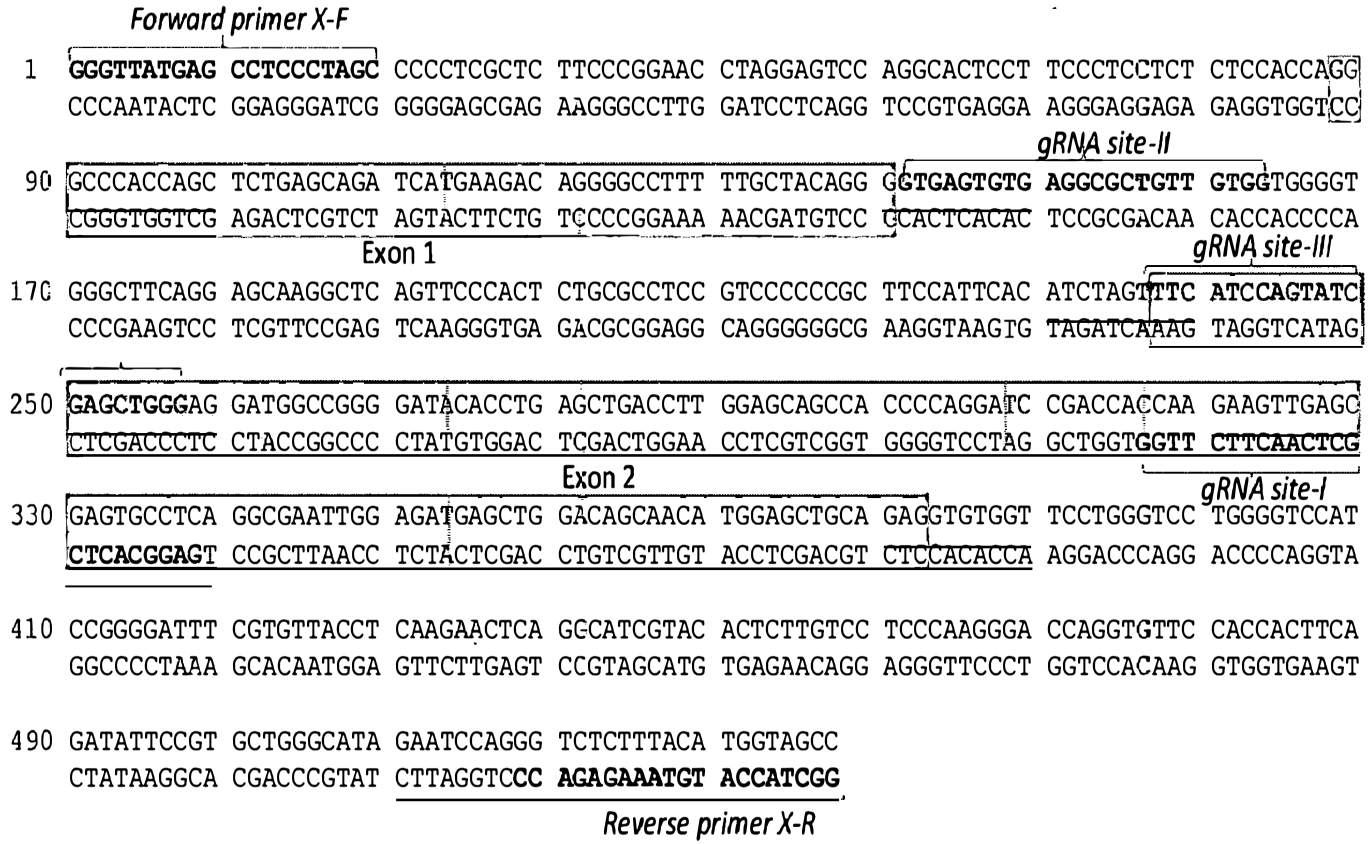

Forward primer X-F
1 GGGTTATGAG CCTCCCTAGC CCCCTCGCTC TTCCCGGAAC CTAGGAGTCC AGGCACTCCT TCCCTCCTCT CTCCACCAGG
CCCAATACTC GGAGGGATCG GGGGAGCGAG AAGGGCCTTG GATCCTCAGG TCCGTGAGGA AGGGAGGAGA GAGGTGGTCC
gRNA site-II
90 GCCCACCAGC TCTGAGCAGA TCATGAAGAC AGGGGCCTTT TTGCTACAGG GGTGAGTGTG AGGCGCTGTT GTGGTGGGGT
CGGGTGGTCG AGACTCGTCT AGTACTTCTG TCCCCGGAAA AACGATGTCC CCACTCACAC TCCGCGACAA CACCACCCCA
Exon 1
gRNA site-III
170 GGGCTTCAGG AGCAAGGCTC AGTTCCCACT CTGCGCCTCC GTCCCCCCGC TTCCATTCAC ATCTAGTTTC ATCCAGTATC
CCCGAAGTCC TCGTTCCGAG TCAAGGGTGA GACGCGGAGG CAGGGGGGCG AAGGTAAGTG TAGATCAAAG TAGGTCATAG
250 GAGCTGGGAG GATGGCCGGG GATACACCTG AGCTGACCTT GGAGCAGCCA CCCCAGGATC CGACCACCAA GAAGTTGAGC
CTCGACCCTC CTACCGGCCC CTATGTGGAC TCGACTGGAA CCTCGTCGGT GGGGTCCTAG GCTGGTGGTT CTTCAACTCG
Exon 2
gRNA site-I
330 GAGTGCCTCA GGCGAATTGG AGATGAGCTG GACAGCAACA TGGAGCTGCA GAGGTGTGGT TCCTGGGTCC TGGGGTCCAT
CTCACGGAGT CCGCTTAACC TCTACTCGAC CTGTCGTTGT ACCTCGACGT CTCCACACCA AGGACCCAGG ACCCCAGGTA
410 CCGGGGATTT CGTGTTACCT CAAGAACTCA GGCATCGTAC ACTCTTGTCC TCCCAAGGGA CCAGGTGTTC CACCACTTCA
GGCCCCTAAA GCACAATGGA GTTCTTGAGT CCGTAGCATG TGAGAACAGG AGGGTTCCCT GGTCCACAAG GTGGTGAAGT
490 GATATTCCGT GCTGGGCATA GAATCCAGGG TCTCTTTACA TGGTAGCC
CTATAAGGCA CGACCCGTAT CTTAGGTCCC AGAGAAATGT ACCATCGG
Reverse primer X-R

FIG. 5

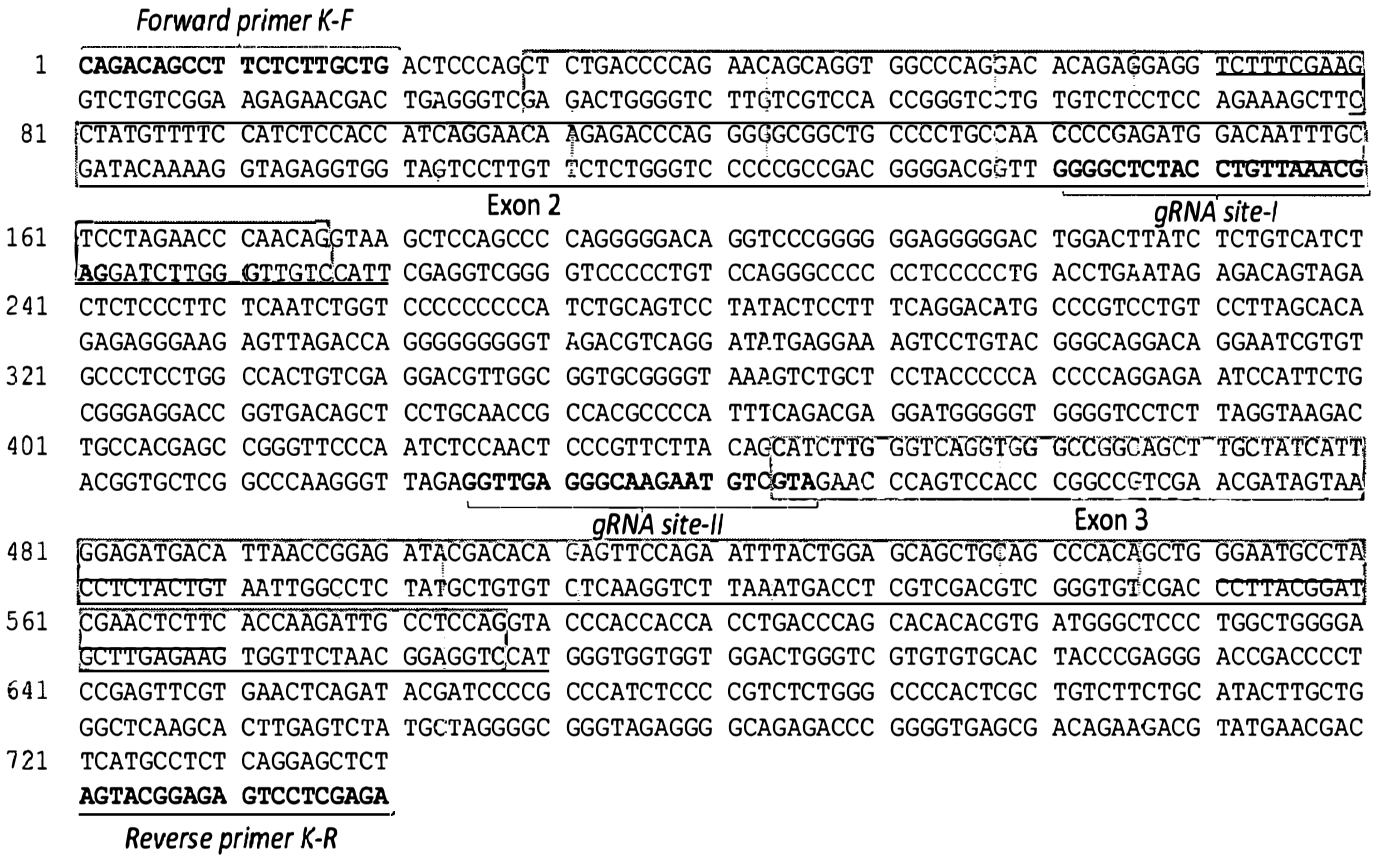
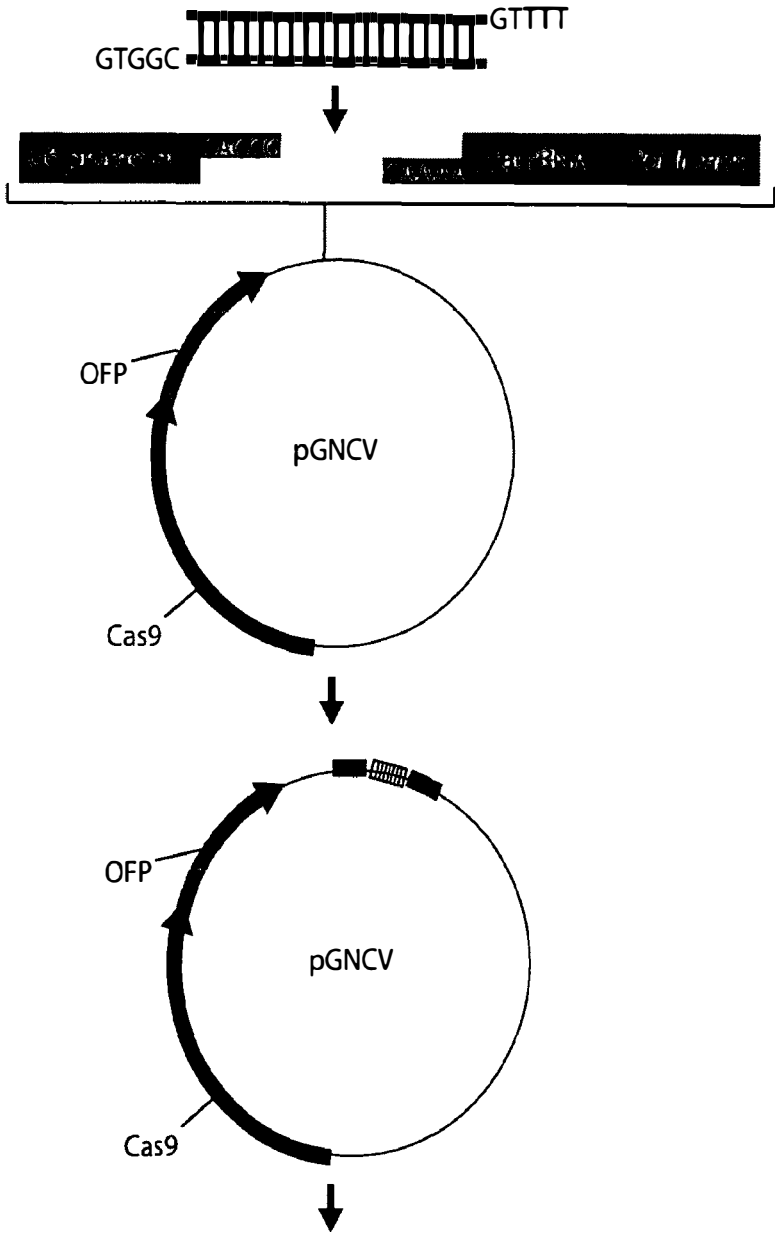

Forward primer K-F
1 CAGACAGCCT TCTCTTGCTG ACTCCCAGCT CTGACCCCAG AACAGCAGGT GGCCCAGGAC ACAGAGGAGG TCTTTCGAAG
GTCTGTCGGA AGAGAACGAC TGAGGGTCGA GACTGGGGTC TTGTCGTCCA CCGGGTCCTG TGTCTCCTCC AGAAAGCTTC
81 CTATGTTTTC CATCTCCACC ATCAGGAACA AGAGACCCAG GGGGCGGCTG CCCCTGCCAA CCCCGAGATG GACAATTTGC
GATACAAAAG GTAGAGGTGG TAGTCCTTGT TCTCTGGGTC CCCCGCCGAC GGGGACGGTT GGGGCTCTAC CTGTTAAACG
Exon 2
gRNA site-I
161 TCCTAGAACC CAACAGGTAA GCTCCAGCCC CAGGGGGACA GGTCCCGGGG GGAGGGGGAC TGGACTTATC TCTGTCATCT
AGGATCTTGG GTTGTCCATT CGAGGTCGGG GTCCCCCTGT CCAGGGCCCC CCTCCCCCTG ACCTGAATAG AGACAGTAGA
241 CTCTCCCTTC TCAATCTGGT CCCCCCCCCA TCTGCAGTCC TATACTCCTT TCAGGACATG CCCGTCCTGT CCTTAGCACA
GAGAGGGAAG AGTTAGACCA GGGGGGGGGT AGACGTCAGG ATATGAGGAA AGTCCTGTAC GGGCAGGACA GGAATCGTGT
321 GCCCTCCTGG CCACTGTCGA GGACGTTGGC GGTGCGGGGT AAAGTCTGCT CCTACCCCCA CCCCAGGAGA ATCCATTCTG
CGGGAGGACC GGTGACAGCT CCTGCAACCG CCACGCCCCA TTTCAGACGA GGATGGGGGT GGGGTCCTCT TAGGTAAGAC
401 TGCCACGAGC CGGGTTCCCA ATCTCCAACT CCCGTTCTTA CAGCATCTTG GGTCAGGTGG GCCGGCAGCT TGCTATCATT
ACGGTGCTCG GCCCAAGGGT TAGAGGTTGA GGGCAAGAAT GTCGTAGAAC CCAGTCCACC CGGCCGTCGA ACGATAGTAA
gRNA site-II
Exon 3
481 GGAGATGACA TTAACCGGAG ATACGACACA GAGTTCCAGA ATTTACTGGA GCAGCTGCAG CCCACAGCTG GGAATGCCTA
CCTCTACTGT AATTGGCCTC TATGCTGTGT CTCAAGGTCT TAAATGACCT CGTCGACGTC GGGTGTCGAC CCTTACGGAT
561 CGAACTCTTC ACCAAGATTG CCTCCAGGTA CCCACCACCA CCTGACCCAG CACACACGTG ATGGGCTCCC TGGCTGGGGA
GCTTGAGAAG TGGTTCTAAC GGAGGTCCAT GGGTGGTGGT GGACTGGGTC GTGTGTGCAC TACCCGAGGG ACCGACCCCT
641 CCGAGTTCGT GAACTCAGAT ACGATCCCCG CCCATCTCCC CGTCTCTGGG CCCCACTCGC TGTCTTCTGC ATACTTGCTG
GGCTCAAGCA CTTGAGTCTA TGCTAGGGGC GGGTAGAGGG GCAGAGACCC GGGGTGAGCG ACAGAAGACG TATGAACGAC
721 TCATGCCTCT CAGGAGCTCT
AGTACGGAGA GTCCTCGAGA
Reverse primer K-R

FIG. 6



transfection, single cell deposition, screening by DNA sequencing

FIG. 7

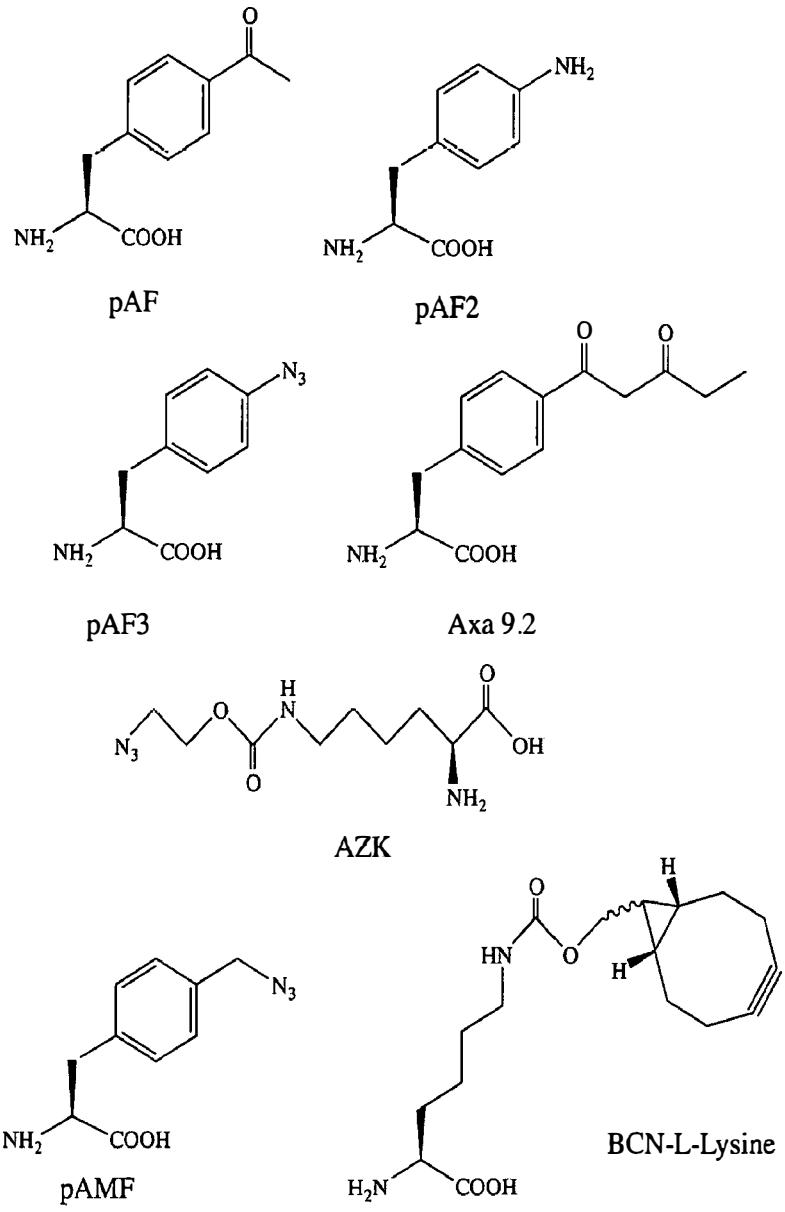


FIG. 8

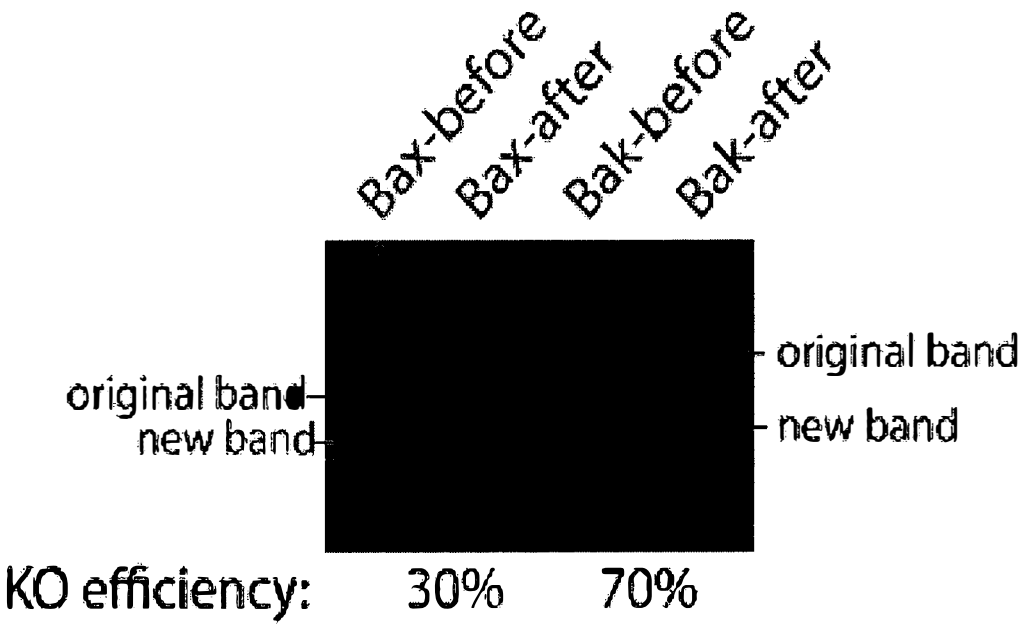

Bax-before
Bax-after
Bak-before
Bak-after
original band
new band
original band
new band
KO efficiency:
30%
70%

FIG. 9

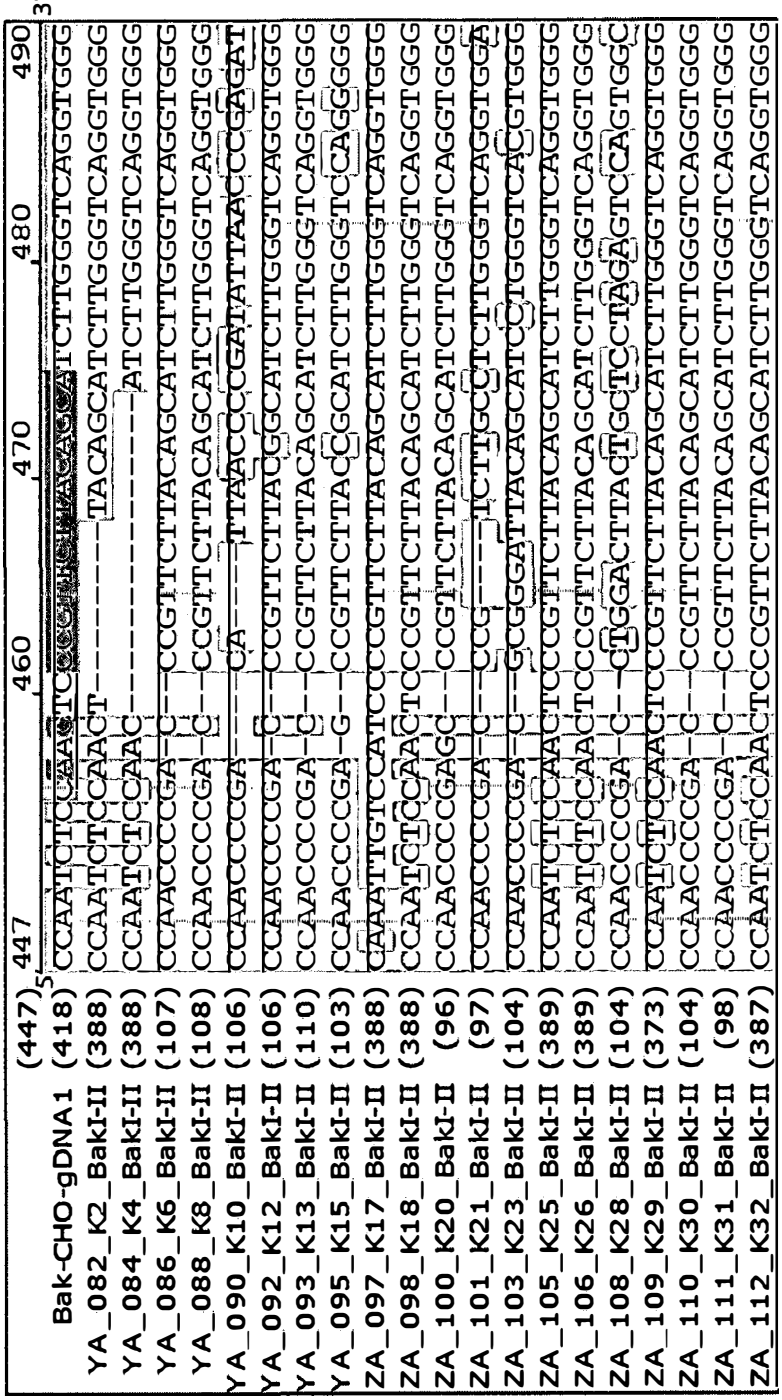

5'
3'
(447)
447
460
470
480
490
Bak-CHO-gDNA1 (418)
YA_082_K2_Bak-II (388)
YA_084_K4_Bak-II (388)
YA_086_K6_Bak-II (107)
YA_088_K8_Bak-II (108)
YA_090_K10_Bak-II (106)
YA_092_K12_Bak-II (106)
YA_093_K13_Bak-II (110)
YA_095_K15_Bak-II (103)
ZA_097_K17_Bak-II (388)
ZA_098_K18_Bak-II (388)
ZA_100_K20_Bak-II (96)
ZA_101_K21_Bak-II (97)
ZA_103_K23_Bak-II (104)
ZA_105_K25_Bak-II (389)
ZA_106_K26_Bak-II (389)
ZA_108_K28_Bak-II (104)
ZA_109_K29_Bak-II (373)
ZA_110_K30_Bak-II (104)
ZA_111_K31_Bak-II (98)
ZA_112_K32_Bak-II (387)

FIG. 10

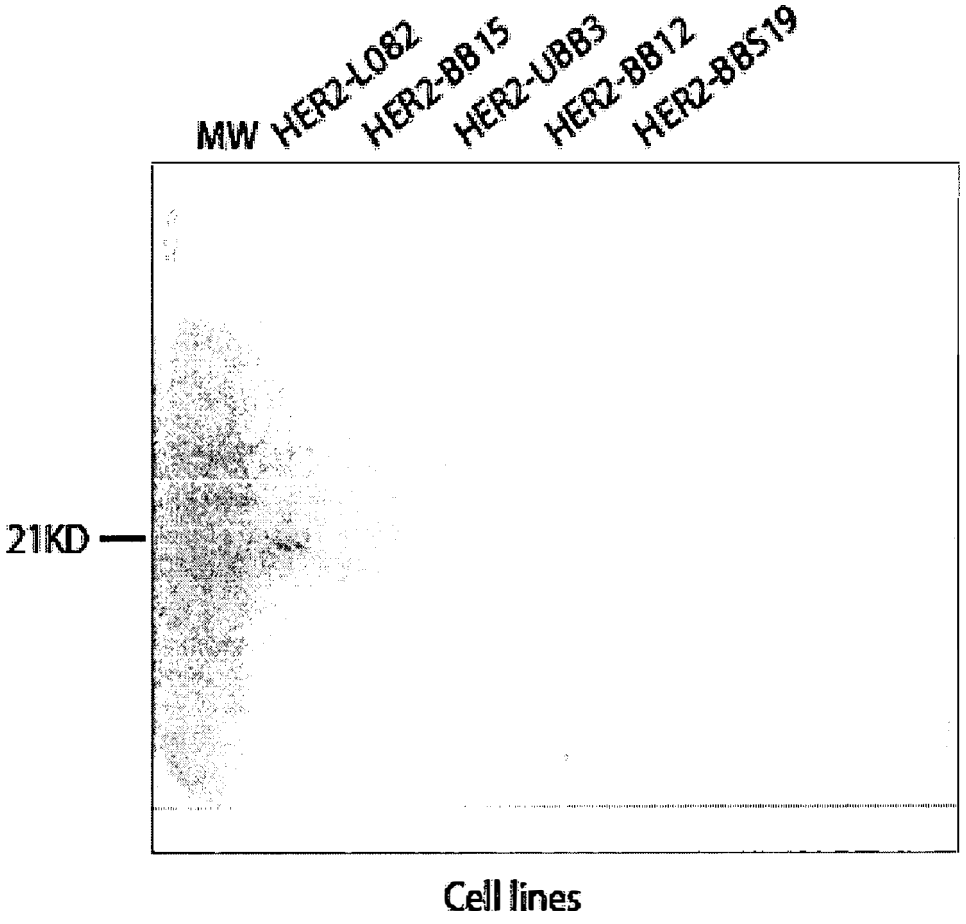
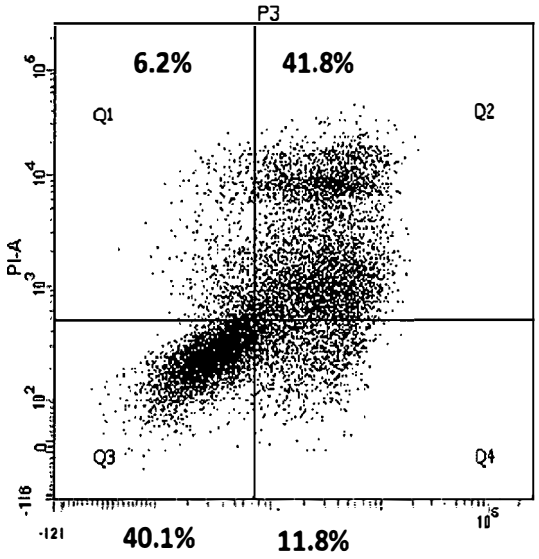
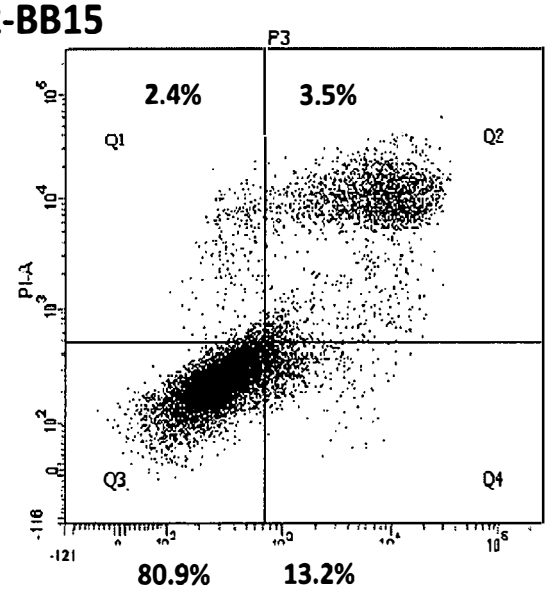

MW
HER2-L082
HER2-BB15
HER2-UBB3
HER2-BB12
HER2-BBS19
21KD
Cell lines

FIG. 11

A. HER2-L082



B. HER2-BB15



Cell Line	Viable Cell (Q3)	Early Apoptotic Cell (Q4)	Late Apoptotic Cell (Q2)	Dead Cell (Q1)
HER2-L082	40.1%	11.8%	41.8%	6.2%
HER2-BB15	80.9%	13.2%	3.5%	2.4%

FIG. 12

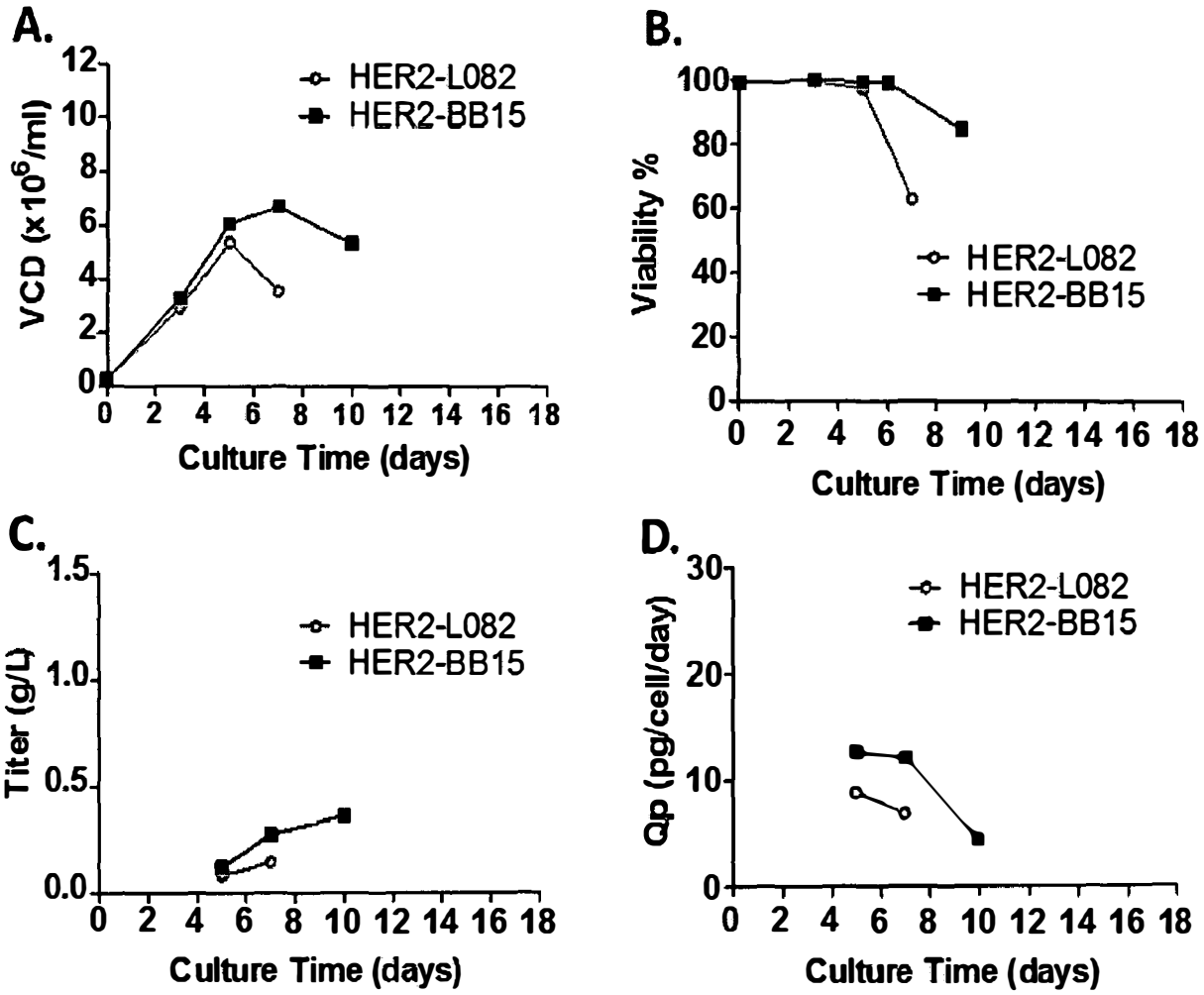

A.
VCD (x10^6/ml)
HER2-L082
HER2-BB15
Culture Time (days)
B.
Viability %
HER2-L082
HER2-BB15
Culture Time (days)
C.
Titer (g/L)
HER2-L082
HER2-BB15
Culture Time (days)
D.
Qp (pg/cell/day)
HER2-L082
HER2-BB15
Culture Time (days)

FIG. 13

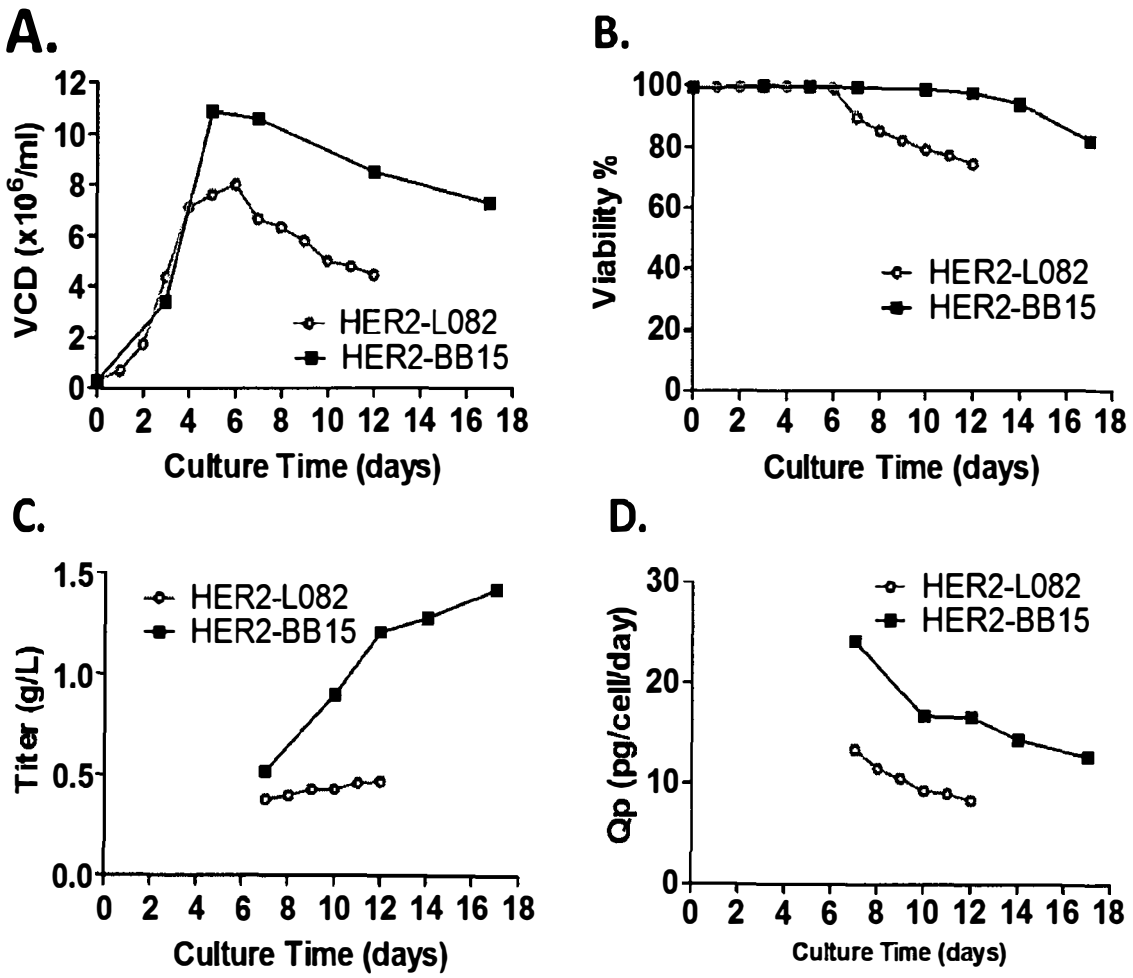

A.
VCD (x10^6/ml)
Culture Time (days)
HER2-L082
HER2-BB15
B.
Viability %
Culture Time (days)
HER2-L082
HER2-BB15
C.
Titer (g/L)
Culture Time (days)
HER2-L082
HER2-BB15
D.
Qp (pg/cell/day)
Culture Time (days)
HER2-L082
HER2-BB15

FIG. 14

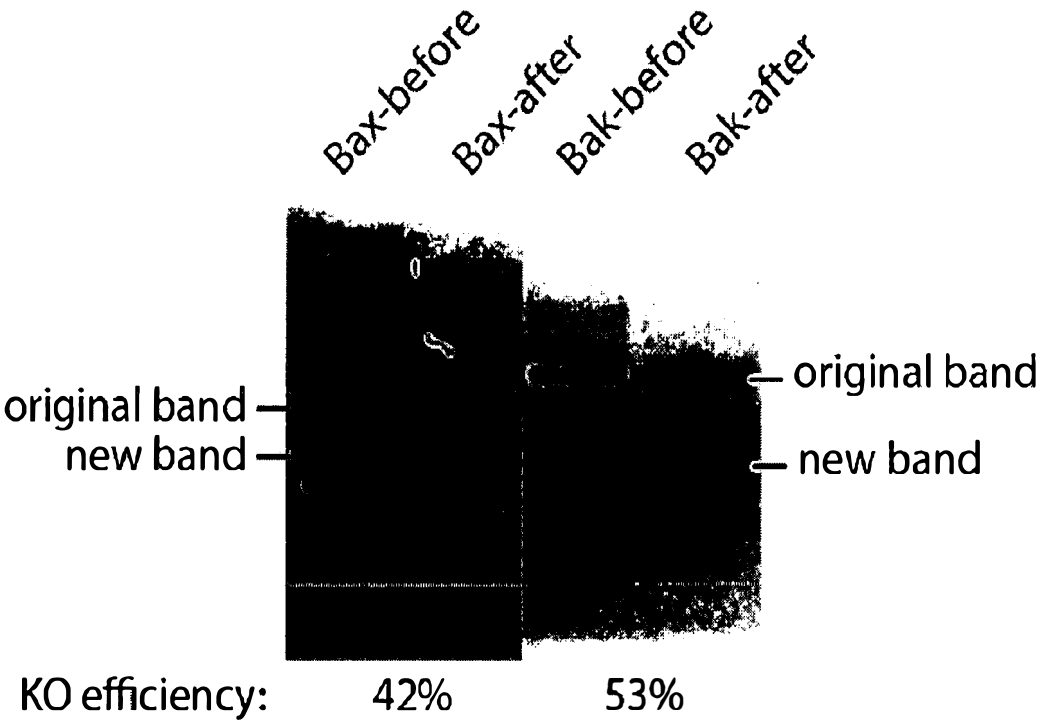
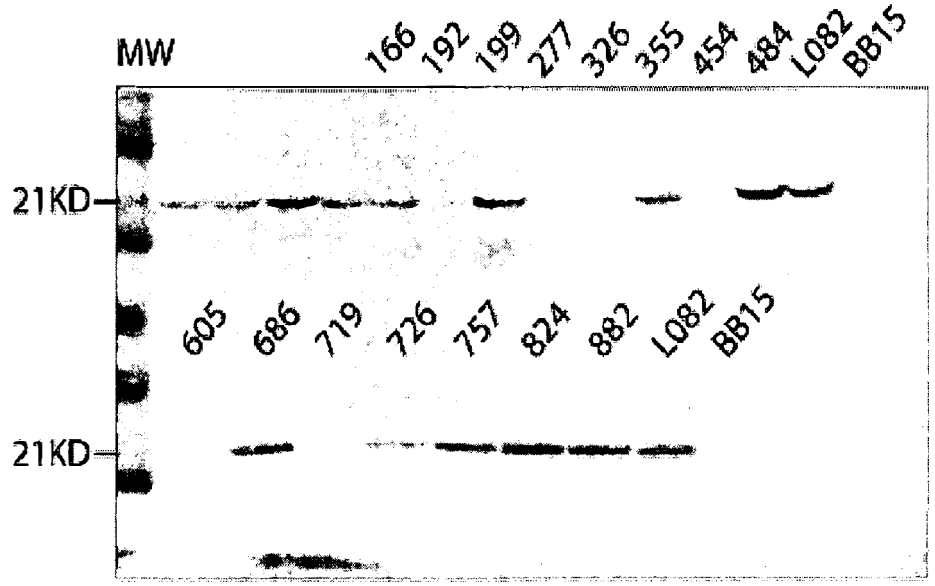

Bax-before
Bax-after
Bak-before
Bak-after
original band
new band
original band
new band
KO efficiency:
42%
53%

FIG. 15

A.


MW
166
192
199
277
326
355
454
484
L082
BB15
21KD
605
686
719
726
757
824
882
L082
BB15
21KD

B.

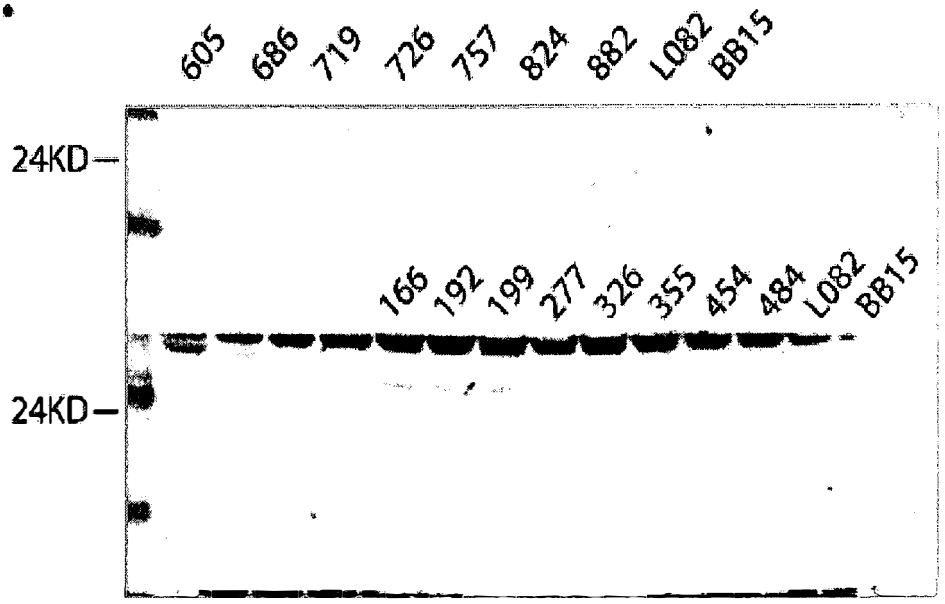
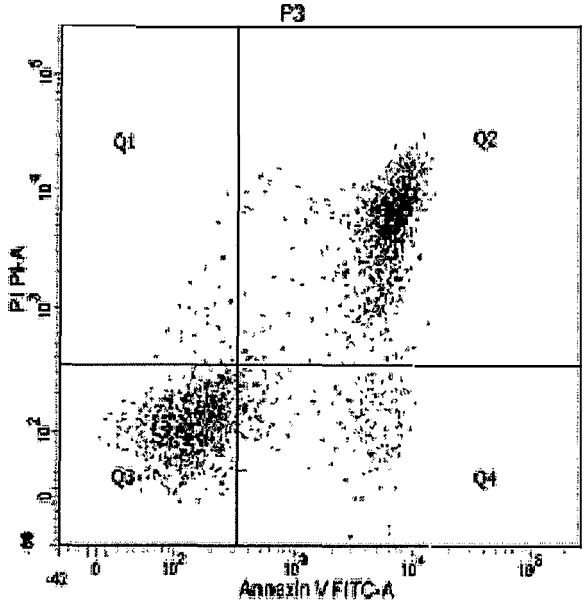
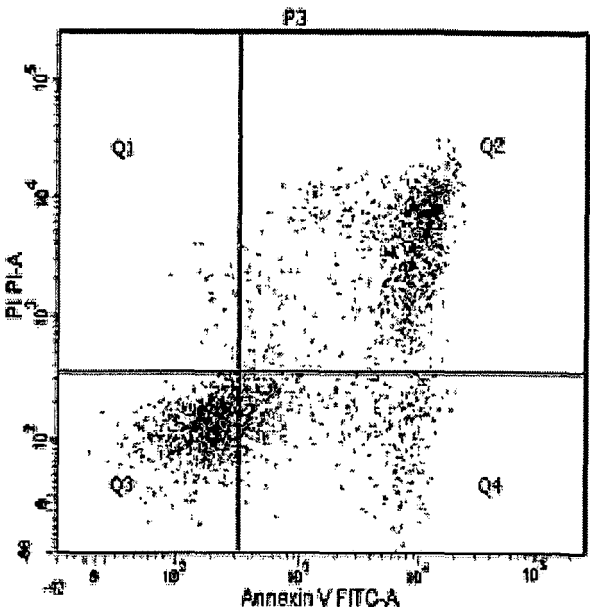

605
686
719
726
757
824
882
L082
BB15
24KD
166
192
199
277
326
355
454
484
L082
BB15
24KD

FIG. 16

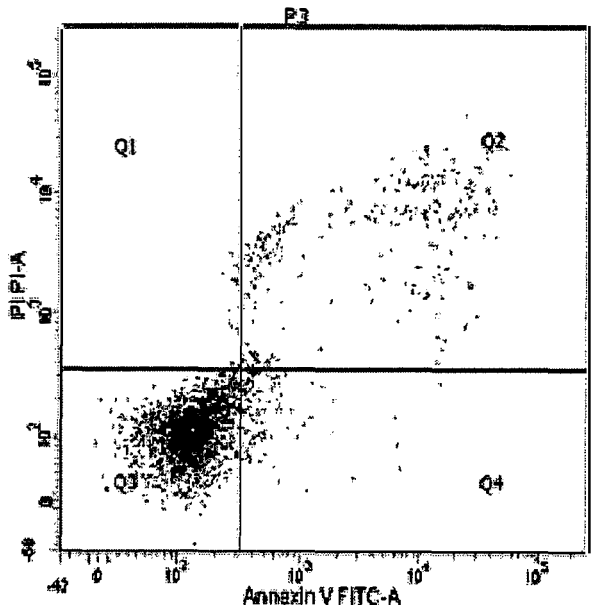
A. PSMA-484



B. PSMA-882



C. PSMA-192



D. PSMA-719

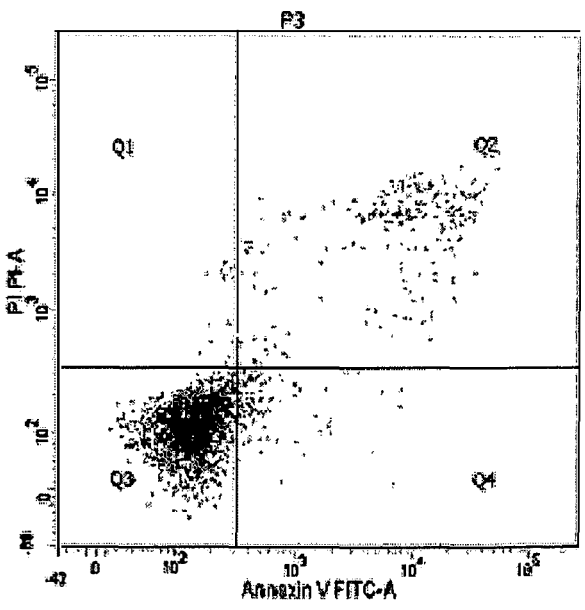


FIG. 17

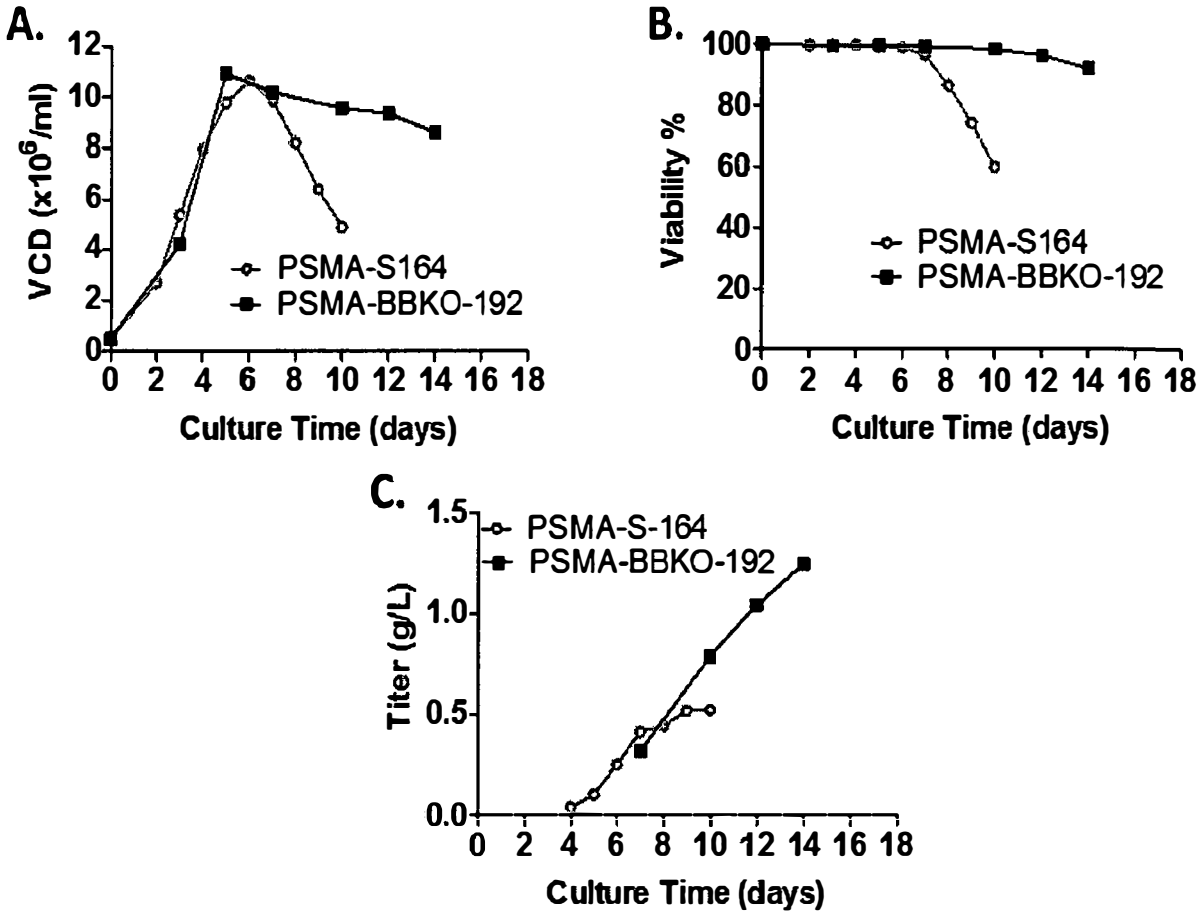

A.
VCD (x10^6/ml)
12
10
8
6
4
2
0
0 2 4 6 8 10 12 14 16 18
Culture Time (days)
PSMA-S164
PSMA-BBKO-192
B.
Viability %
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18
Culture Time (days)
PSMA-S164
PSMA-BBKO-192
C.
Titer (g/L)
1.5
1.0
0.5
0.0
0 2 4 6 8 10 12 14 16 18
Culture Time (days)
PSMA-S-164
PSMA-BBKO-192

FIG. 18

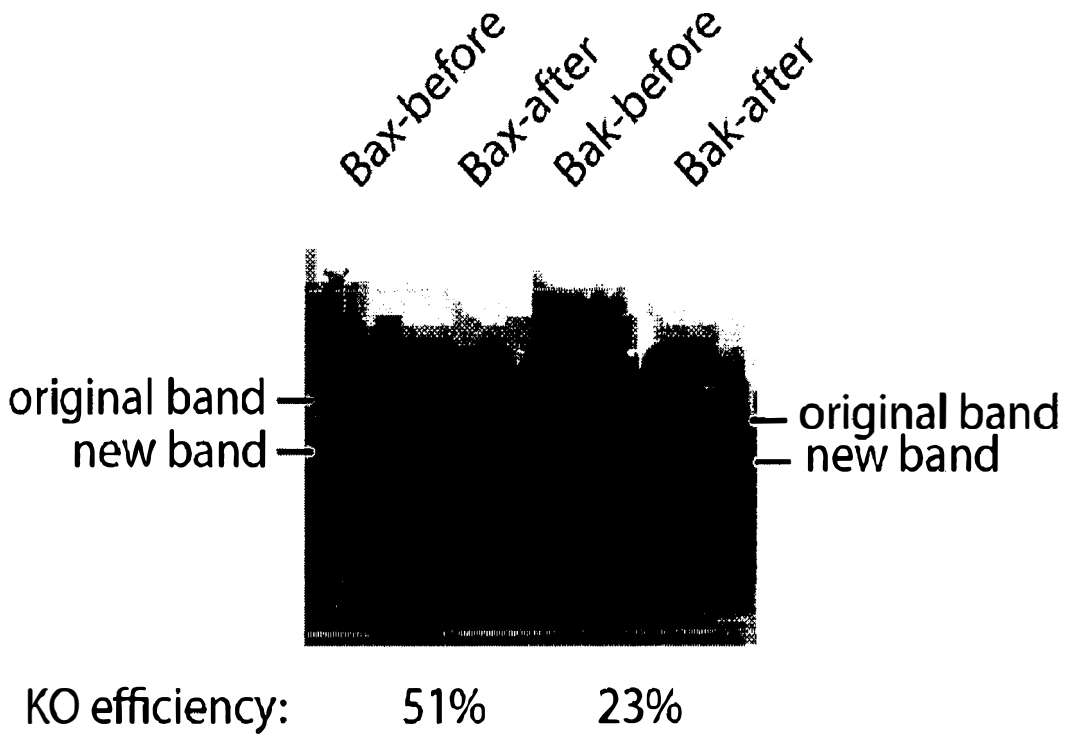

Bax-before
Bax-after
Bak-before
Bak-after
original band
new band
original band
new band
KO efficiency:
51%
23%

FIG. 19

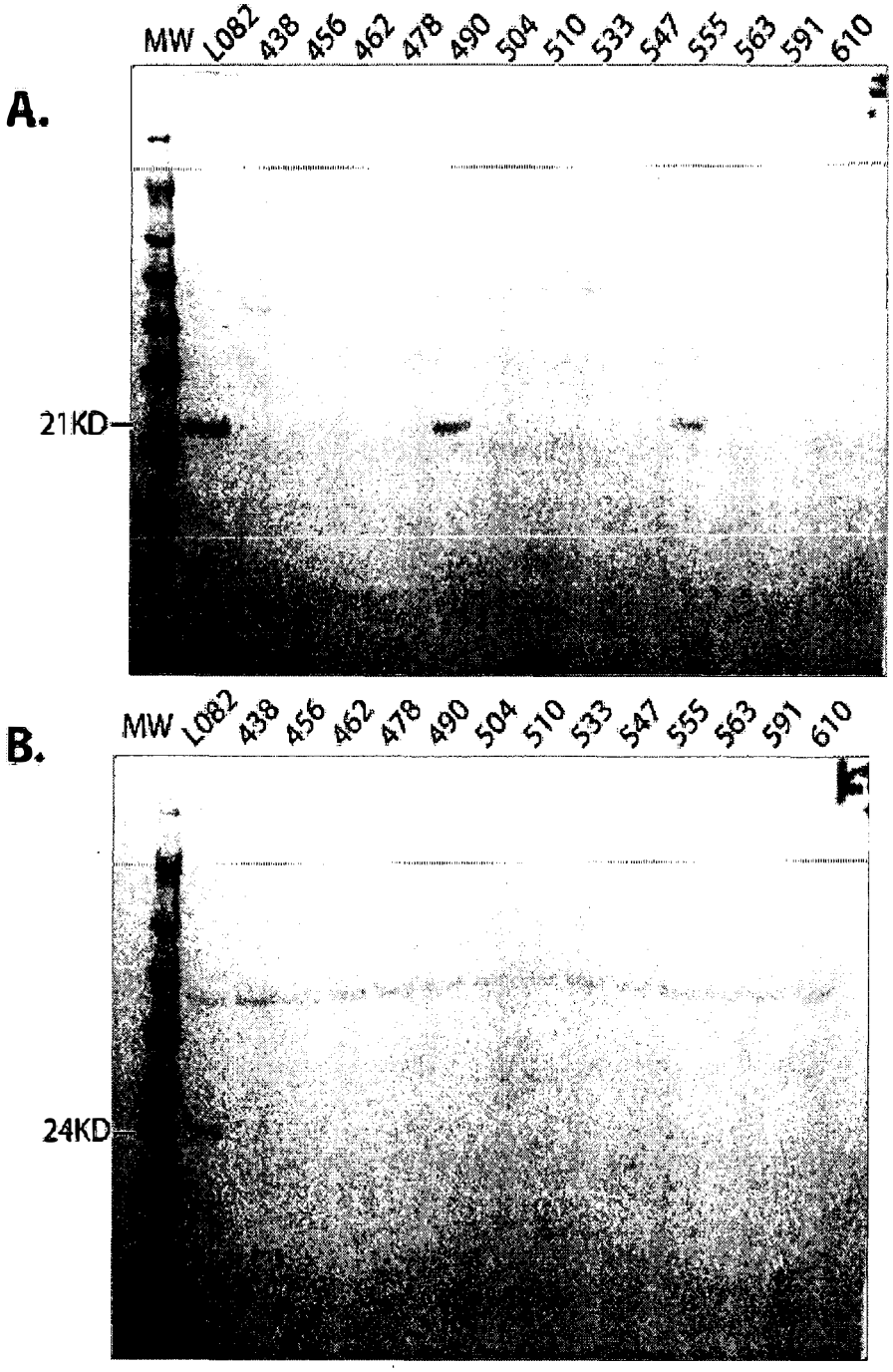
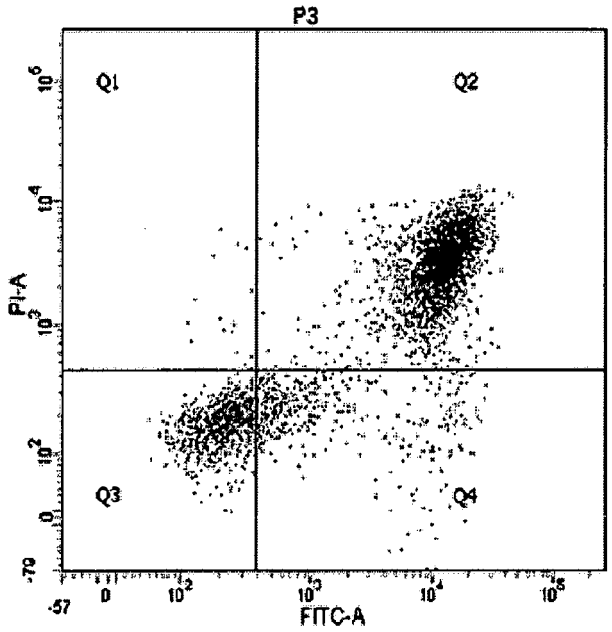
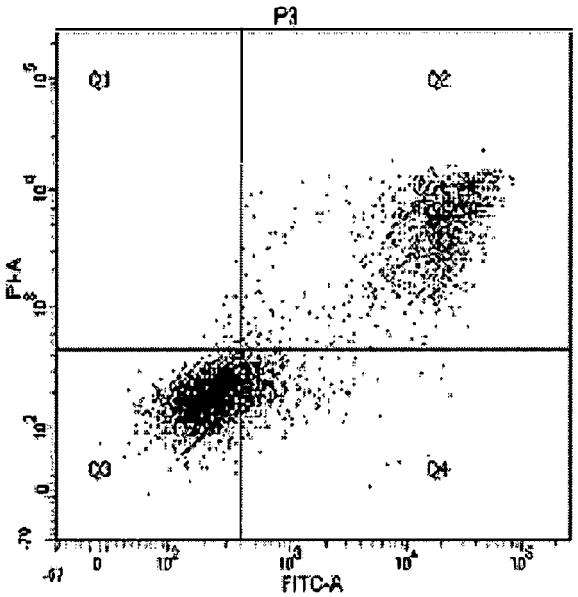

A.
MW
L082
438
456
462
478
490
504
510
533
547
555
563
591
610
21KD
B.
MW
L082
438
456
462
478
490
504
510
533
547
555
563
591
610
24KD

FIG. 20

A. CD70-MW-108



B. CD70-BBKO-563



Cell line	Viable cells (Q3)	Early Apoptotic Cells (Q4)	Late Apoptotic Cells (Q2)	Dead Cells (Q1)
CD70-MW-108	18.3 %	8.8 %	72.5 %	0.3 %
CD70-BBKO-563	60.3 %	11.4 %	27.9 %	0.5 %

FIG. 21

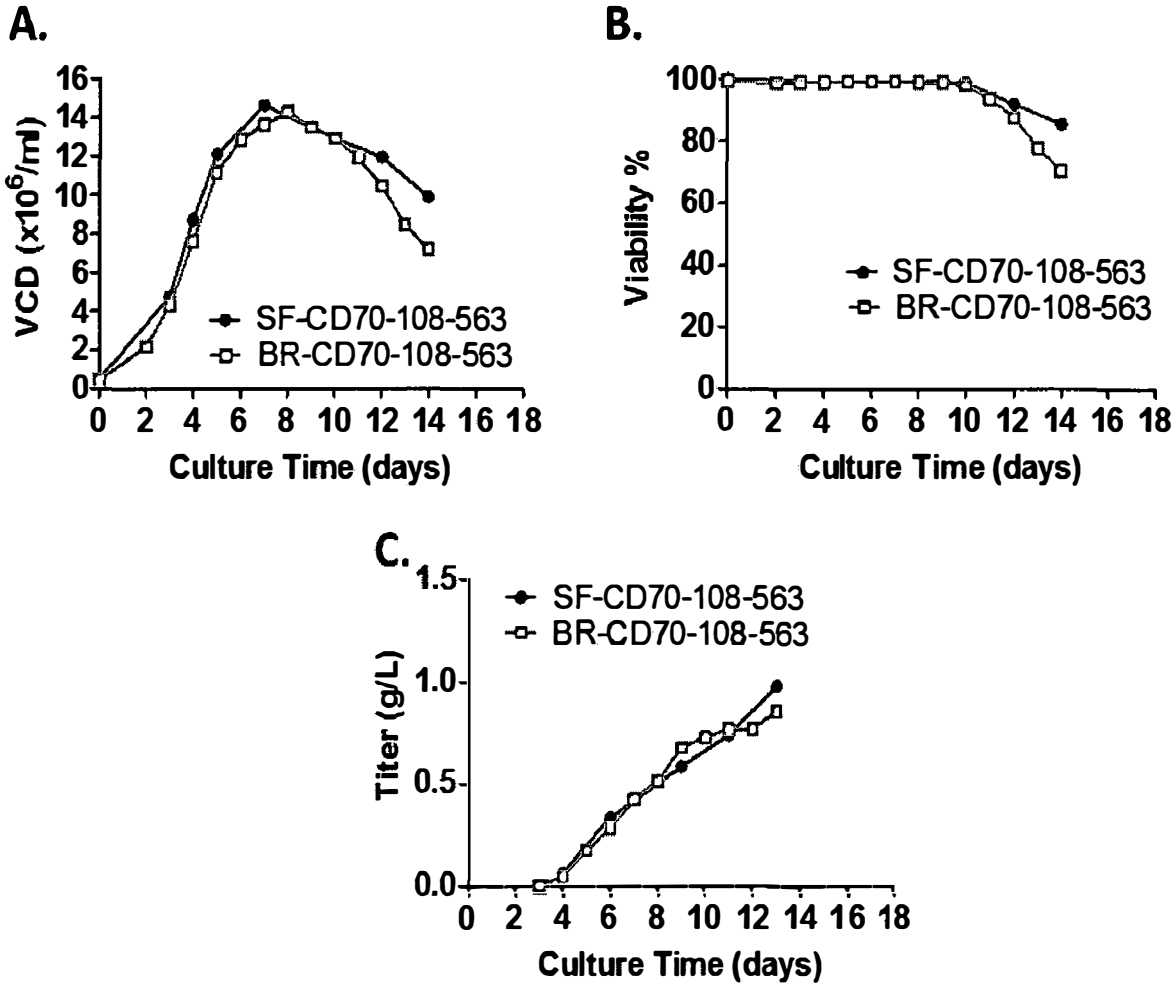

A.
VCD (x10^6/ml)
16
14
12
10
8
6
4
2
0
0 2 4 6 8 10 12 14 16 18
Culture Time (days)
SF-CD70-108-563
BR-CD70-108-563
B.
Viability %
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18
Culture Time (days)
SF-CD70-108-563
BR-CD70-108-563
C.
Titer (g/L)
1.5
1.0
0.5
0.0
0 2 4 6 8 10 12 14 16 18
Culture Time (days)
SF-CD70-108-563
BR-CD70-108-563

FIG. 22