```
MSIVMTQAAPSIPVTPGESVSISCR      25
SSKSLLNSNGNTYLYWFLQRPGQSP      50
QLLIYRMSNLASGVPDRFSGSGSGT      75
AFTLRISRVEAEDVGVYYCMQHLEY     100
PLTFGCGTKLEIKRGGGGSGGGGSG     125
GGGSGGGGSQVQLQQSGPELIKPGA     150
SVKMSCKASGYTFTSYVMHWVKQKP     175
GQCLEWIGYINPYNDGTKYNEKFKG     200
KATLTSDKSSSTAYMELSSLTSEDS     225
AVYYCARGTYYYGSRVFDYWGQGTT     250
LTVTVSSASGAGTGTCYDYKDHDGK     275
DHDIDYKDDDDK*SR               290
```

FIG. 1

Exotoxin A domain III

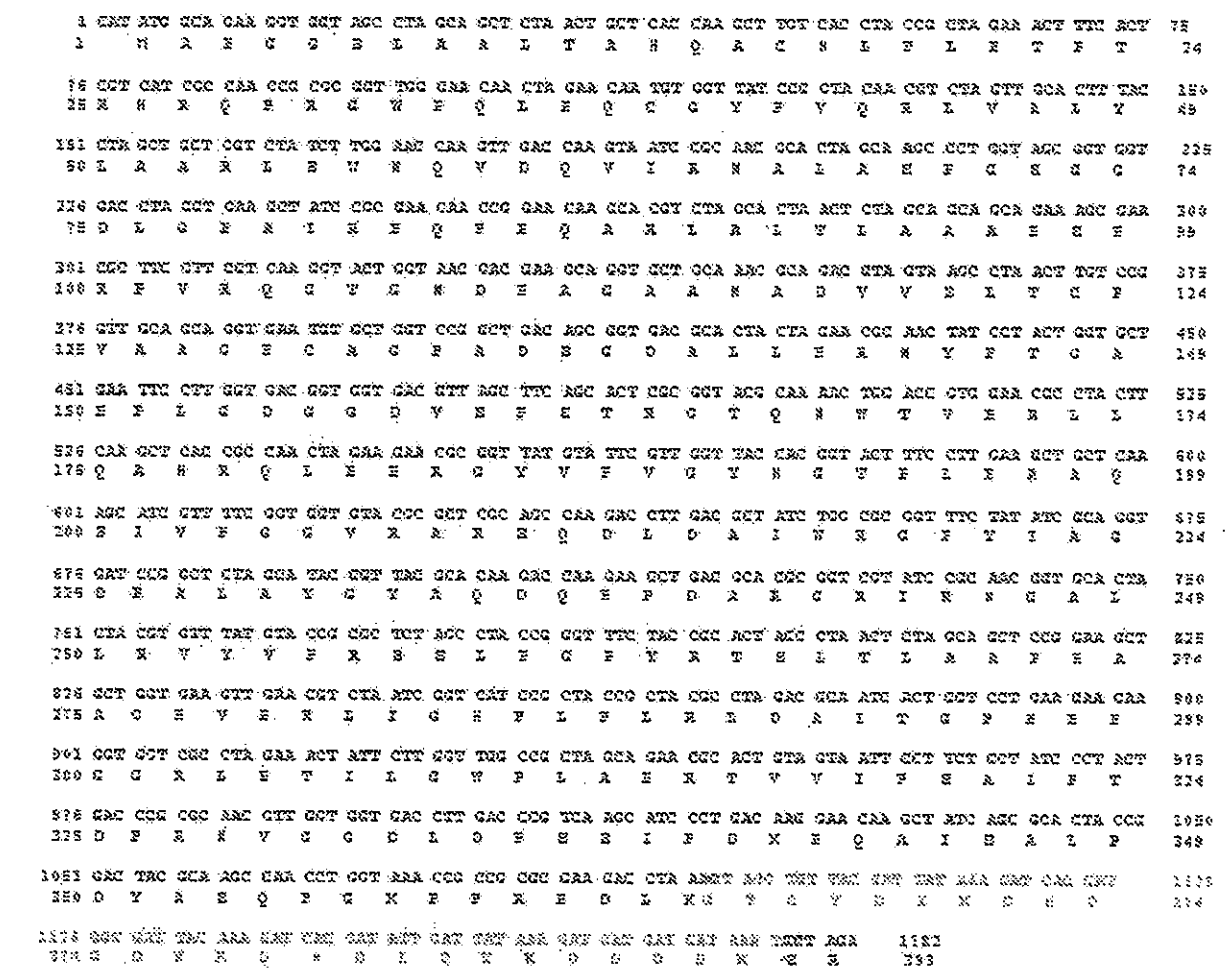


FIG. 2

```
   1 CAT ATG ATA GTT ATG ACA CAA GCT GCA CCA TCT ATT CCT GTT ACT CCT GGA GAA TCA GTA TCA ATC TCA TGT CGT
   1     M   I   V   M   T   Q   A   A   P   S   I   P   V   T   P   G   E   S   V   S   I   S   C   R

  76 TCT AGT AAA AGT CTT CTG AAT AGT AAT GGT AAC ACT TAC TTA TAT TGG TTC CTG CAA CGT CCA GGC CAA TCT CCT
  25 S   S   K   S   L   L   N   S   N   G   N   T   Y   L   Y   W   F   L   Q   R   P   G   Q   S   P

 151 CAA CTT CTG ATT TAT CGT ATG TCA AAC CTT GCT TCA GGT GTT CCA GAC CGT TTC AGT GGT AGT GGT TCA GGA ACT
  50 Q   L   L   I   Y   R   M   S   N   L   A   S   G   V   P   D   R   F   S   G   S   G   S   G   T

 226 GCT TTC ACA CTG AGA ATC AGT AGA GTA GAA GCT GAA GAT GTA GGT GTT TAT TAC TGT ATG CAA CAT TTA GAA TAT
  75 A   F   T   L   R   I   S   R   V   E   A   E   D   V   G   V   Y   Y   C   M   Q   H   L   E   Y

 301 CCT CTT ACT TTC GGT TGT GGT ACA AAA CTG GAA ATC AAA CGA GGA GGA GGA GGA TCT GGA GGA GGA GGA TCT GGA
 100 P   L   T   F   G   C   G   T   K   L   E   I   K   R   G   G   G   G   S   G   G   G   G   S   G

 376 GGA GGC GGA TCA GGA GGA GGT GGT TCA CAA GTT CAG CTT CAA CAA TCT GGA CCT GAA CTG ATT AAA CCT GGT GCT
 125 G   G   G   S   G   G   G   G   S   Q   V   Q   L   Q   Q   S   G   P   E   L   I   K   P   G   A

 451 TCA GTA AAA ATG TCA TGT AAA GCT TCT GGA TAC ACA TTC ACT AGC TAT GTT ATG CAC TGG GTA AAA CAA AAA CCT
 150 S   V   K   M   S   C   K   A   S   G   Y   T   F   T   S   Y   V   M   H   W   V   K   Q   K   P

 526 GGT CAA TGT CTT GAA TGG ATT GGA TAT ATT AAT CCT TAC AAT GAT GGT ACT AAA TAC AAT GAA AAA TTC AAA GGT
 175 G   Q   C   L   E   W   I   G   Y   I   N   P   Y   N   D   G   T   K   Y   N   E   K   F   K   G

 601 AAA GCT ACA CTG ACT TCA GAC AAA TCA TCA AGC ACA GCT TAC ATG GAA CTT AGC AGC CTG ACA TCT GAA GAC TCT
 200 K   A   T   L   T   S   D   K   S   S   S   T   A   Y   M   E   L   S   S   L   T   S   E   D   S

 676 GCA GTT TAT TAC TGT GCA AGA GGT ACT TAT TAC TAC GGT AGT CGT GTA TTT GAC TAC TGG GGC CAA GGT ACA ACT
 225 A   V   Y   Y   C   A   R   G   T   Y   Y   Y   G   S   R   V   F   D   Y   W   G   Q   G   T   T

 751 CTT ACA GTT ACA GTT TCA TCT GCT TCT GGT CCT GGT AGC AGT TCT GGT GGC GGT GGC AGT AGT GGT GGT GGC GGT
 250 L   T   V   T   V   S   S   A   S   G   A   G   T   S   S   G   G   G   G   S   S   G   G   S   G

 826 AGT AGT GGT GGC GGT GGC ATG GCA GAA GGT GGT AGC CTA GCA GCT CTA ACT GCT CAC CAA GCT TGT CAC CTA CCG
 275 S   S   G   G   G   G   M   A   E   G   G   S   L   A   A   L   T   A   H   Q   A   C   H   L   P

 901 CTA GAA ACT TTC ACT CGT CAT CGC CAA CCG CGC GGT TGG GAA CAA CTA GAA CAA TGT GGT TAT CCG GTA CAA CGT
 300 L   E   T   F   T   R   H   R   Q   P   R   G   W   E   Q   L   E   Q   C   G   Y   P   V   Q   R

 976 CTA GTT GCA CTT TAC CTA GCT GCT CGT CTA TCT TGG AAC CAA GTT GAC CAA GTA ATC CGC AAC GCA CTA GCA AGC
 325 L   V   A   L   Y   L   A   A   R   L   S   W   N   Q   V   D   Q   V   I   R   N   A   L   A   S

1051 CCT GGT AGC GGT GGT GAC CTA GGT GAA GCT ATC CGC GAA CAA CCG GAA CAA GCA CGT CTA GCA CTA ACT CTA GCA
 350 P   G   S   G   G   D   L   G   E   A   I   R   E   Q   P   E   Q   A   R   L   A   L   T   L   A

1126 GCA GCA GAA AGC GAA CGC TTC GTT CGT CAA GGT ACT GGT AAC GAC GAA GCA GGT GCT GCA AAC GCA GAC GTA GTA
 375 A   A   E   S   E   R   F   V   R   Q   G   T   G   N   D   E   A   G   A   N   A   D   V   V

1201 AGC CTA ACT TGT CCG GTT GCA GCA GGT GAA TGT GCT GGT CCG GCT GAC AGC GGT GAC GCA CTA CTA GAA CGC AAC
 400 S   L   T   C   P   V   A   A   G   E   C   A   G   P   A   D   S   G   D   A   L   L   E   R   N

1276 TAT CCT ACT GGT GCT GAA TTC CTT GGT GAC GGT GGT GAC GTT AGC TTC AGC ACT CGC GGT ACG CAA AAC TGG ACC
 425 Y   P   T   G   A   E   F   L   G   D   G   G   D   V   S   F   S   T   R   G   T   Q   N   W   T

1351 GTG GAA CGC CTA CTT CAA GCT CAC CGC CAA CTA GAA GAA CGC GGT TAT GTA TTC GTT GGT TAC CAC GGT ACT TTC
 450 V   E   R   L   L   Q   A   H   R   Q   L   E   E   R   G   Y   V   F   V   G   Y   H   G   T   F

1426 CTT GAA GCT GCT CAA AGC ATC GTT TTC GGT GGT GTA CGC GCT CGC AGC CAA GAC CTT GAC GCT ATC TGG CGC GGT
 475 L   E   A   A   Q   S   I   V   F   G   G   V   R   A   R   S   Q   D   L   D   A   I   W   R   G
```

FIG. 3

```
1501 TTC TAT ATC GCA GGT GAT CCG GCT CTA GCA TAC GGT TAC GCA CAA GAC CAA GAA CCT GAC GCA CGC GGT CGT ATC
 500  F   Y   I   A   G   D   P   A   L   A   Y   G   Y   A   Q   D   Q   E   P   D   A   R   G   R   I

1576 CGC AAC GGT GCA CTA CTA CGT GTT TAT GTA CCG CGC TCT AGC CTA CCG GGT TTC TAC CGC ACT AGC CTA ACT CTA
 525  R   N   G   A   L   L   R   V   Y   V   P   R   S   S   L   P   G   F   Y   R   T   S   L   T   L

1651 GCA GCT CCG GAA GCT GCT GGT GAA GTT GAA CGT CTA ATC GGT CAT CCG CTA CCG CTA CGC CTA GAC GCA ATC ACT
 550  A   A   P   E   A   A   G   E   V   E   R   L   I   G   H   P   L   P   L   R   L   D   A   I   T

1726 GGT CCT GAA GAA GAA GGT GGT CGC CTA GAA ACT ATT CTT GGT TGG CCG CTA GCA GAA CGC ACT GTA GTA ATT CCT
 575  G   P   E   E   E   G   G   R   L   E   T   I   L   G   W   P   L   A   E   R   T   V   V   I   P

1801 TCT GCT ATC CCT ACT GAC CCG CGC AAC GTT GGT GGT GAC CTT GAC CCG TCA AGC ATC CCT GAC AAG GAA CAA GCT
 600  S   A   I   P   T   D   P   R   N   V   G   G   D   L   D   P   S   S   I   P   D   K   E   Q   A

1876 ATC AGC GCA CTA CCG GAC TAC GCA AGC CAA CCT GGT AAA CCG CCG CGC GAA GAC CTA AAG GGT ACA TGT TGC GAT
 625  I   S   A   L   P   D   Y   A   S   Q   P   G   K   P   P   R   E   D   L   K   G   T   C   F   D

1951 TAT AAA GAT CAC GAT GGT GAT TAC AAA GAT CAC GAT ATT GAT TAT AAA GAT GAT GAT GAT AAA TAA TCTAGA
 650  Y   K   D   H   D   G   D   Y   K   D   H   D   I   D   Y   K   D   D   D   D   K   *
```

FIG. 3 (Cont'd.)

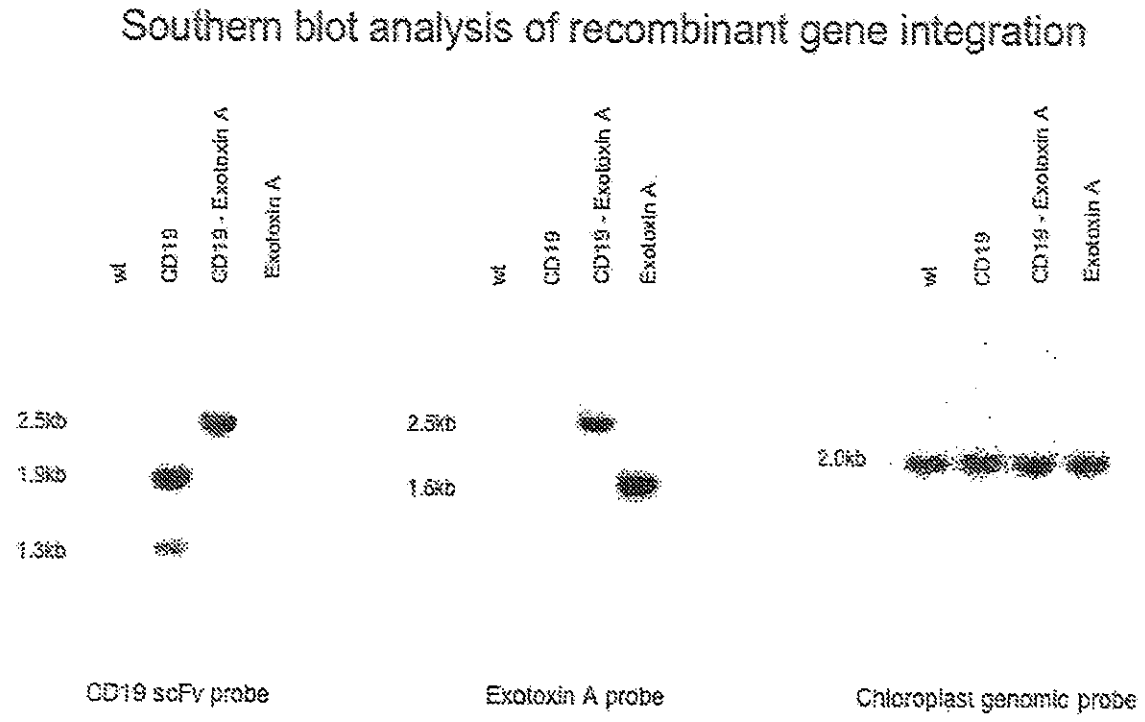


FIG. 4

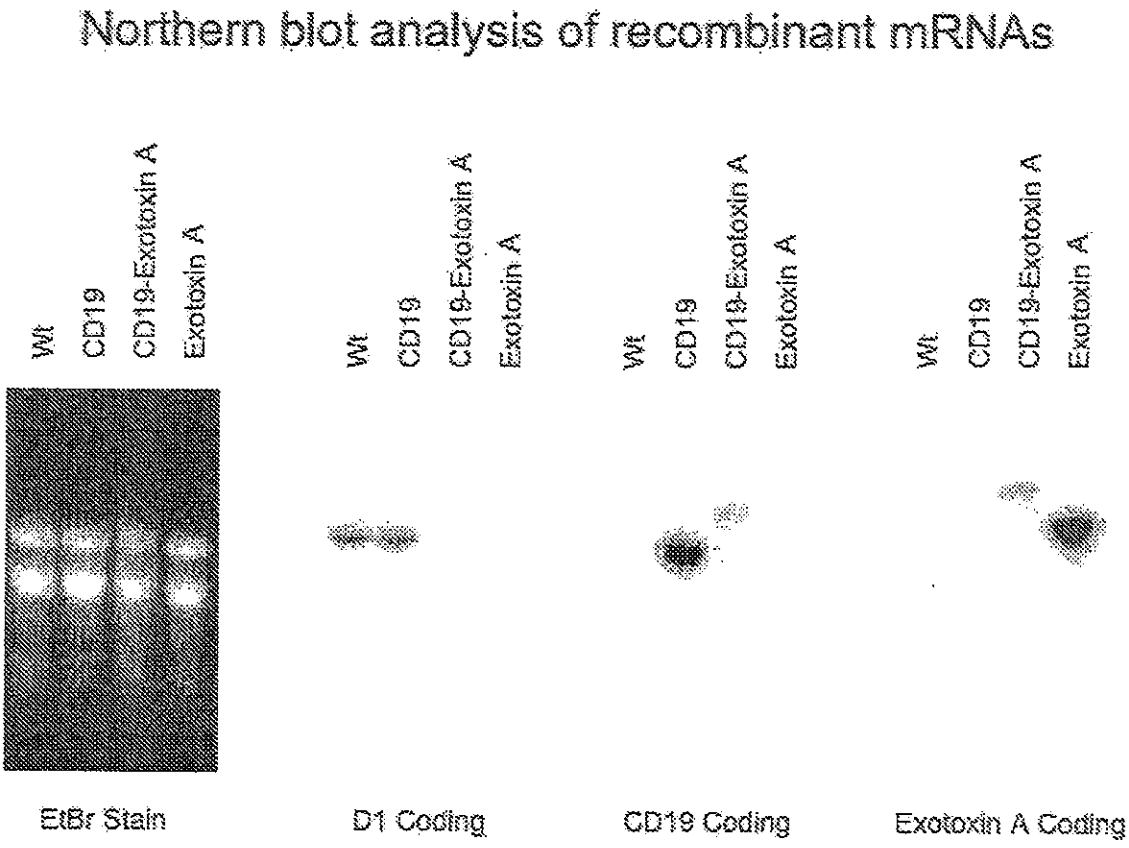

Northern blot analysis of recombinant mRNAs
Wt
CD19
CD19-Exotoxin A
Exotoxin A
EtBr Stain
D1 Coding
CD19 Coding
Exotoxin A Coding

FIG. 5

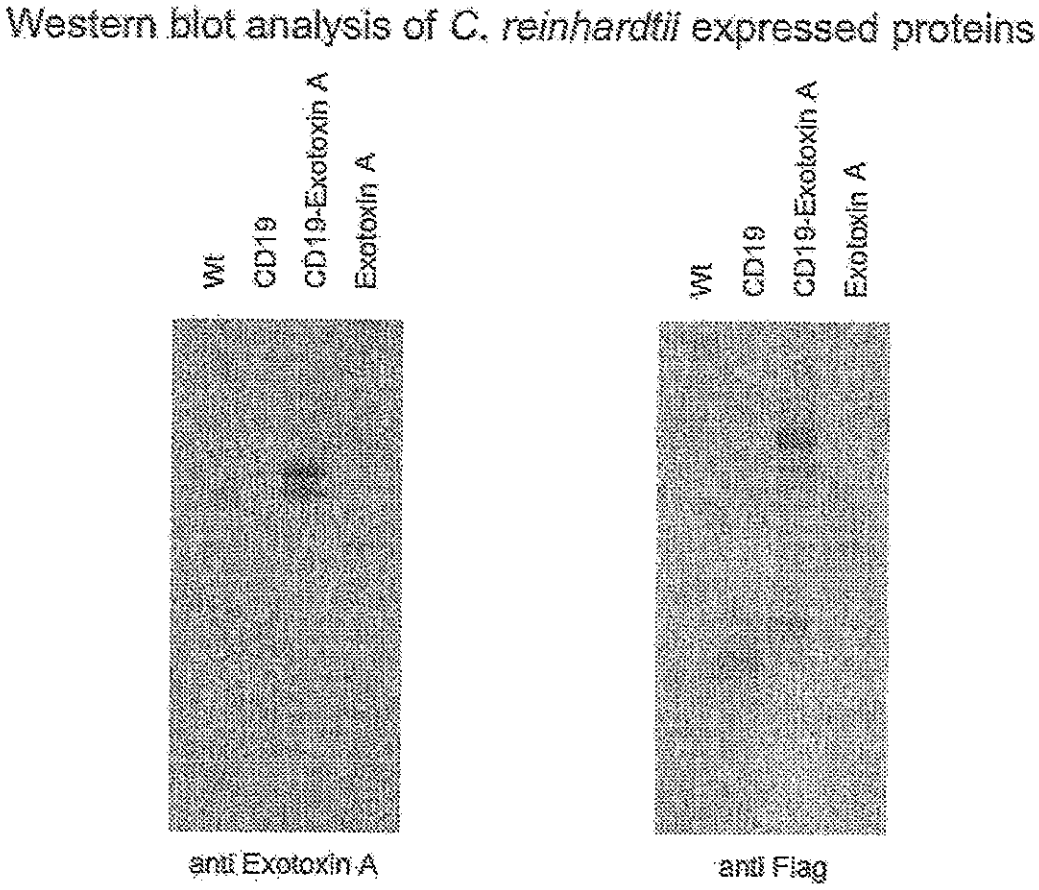

Western blot analysis of C. reinhardtii expressed proteins
Wt
CD19
CD19-Exotoxin A
Exotoxin A
anti Exotoxin A
Wt
CD19
CD19-Exotoxin A
Exotoxin A
anti Flag

FIG. 6

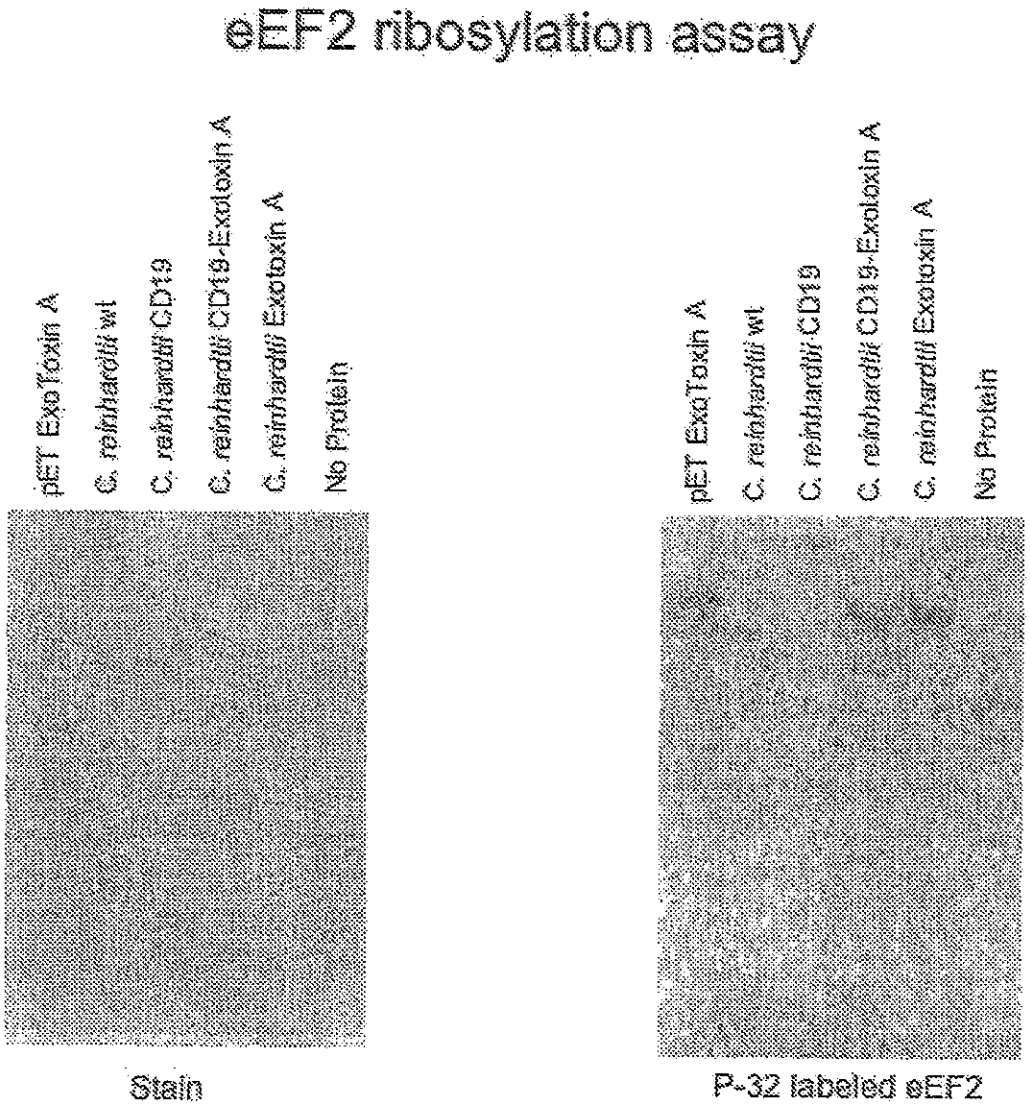

eEF2 ribosylation assay
pET ExoToxin A
C. reinhardtii wt
C. reinhardtii CD19
C. reinhardtii CD19-Exotoxin A
C. reinhardtii Exotoxin A
No Protein
Stain
P-32 labeled eEF2

FIG. 7

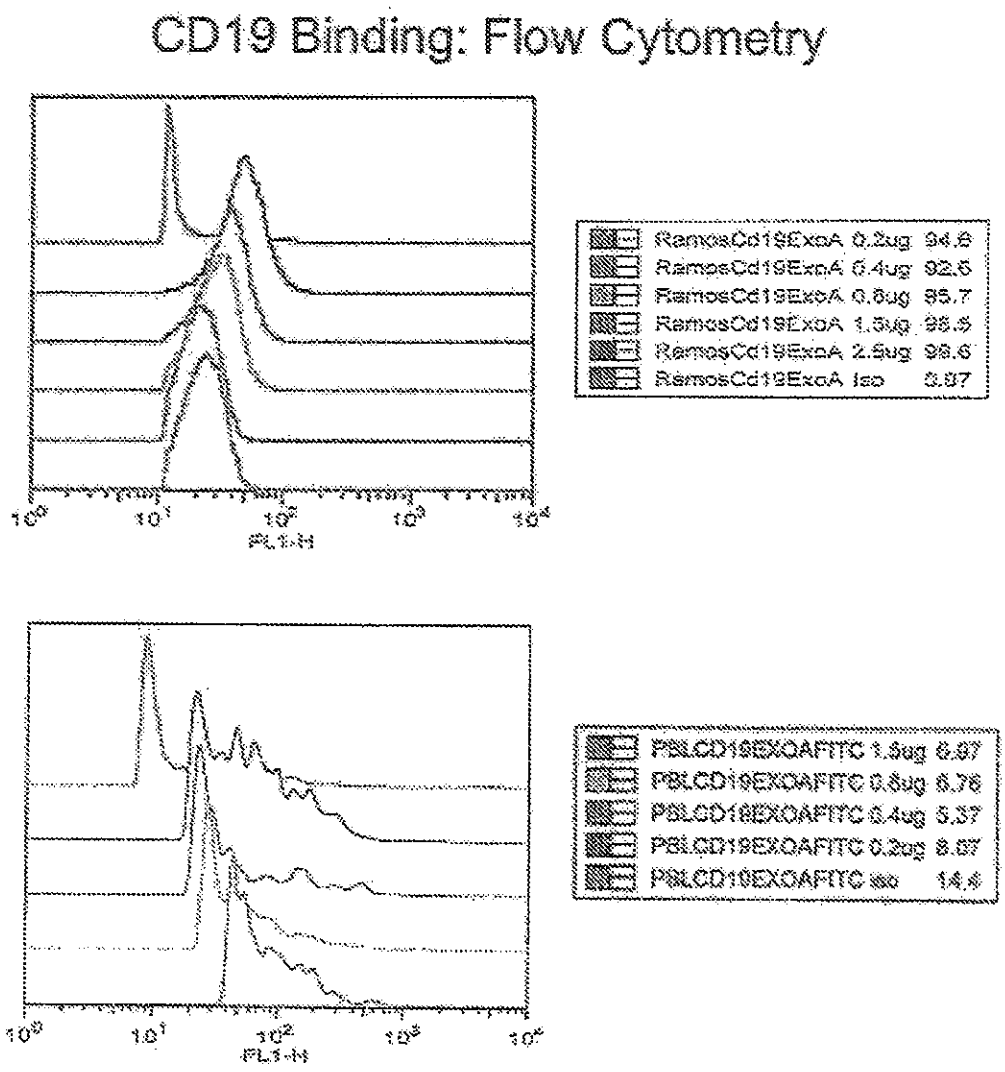

CD19 Binding: Flow Cytometry
RamosCd19ExoA 0.2ug 94.8
RamosCd19ExoA 0.4ug 92.5
RamosCd19ExoA 0.8ug 85.7
RamosCd19ExoA 1.5ug 95.5
RamosCd19ExoA 2.5ug 98.6
RamosCd19ExoA iso 0.87
FL1-H
PBLCD19EXOAFITC 1.5ug 6.87
PBLCD19EXOAFITC 0.8ug 6.76
PBLCD19EXOAFITC 0.4ug 5.37
PBLCD19EXOAFITC 0.2ug 8.67
PBLCD19EXOAFITC iso 14.6
FL1-H

FIG. 8

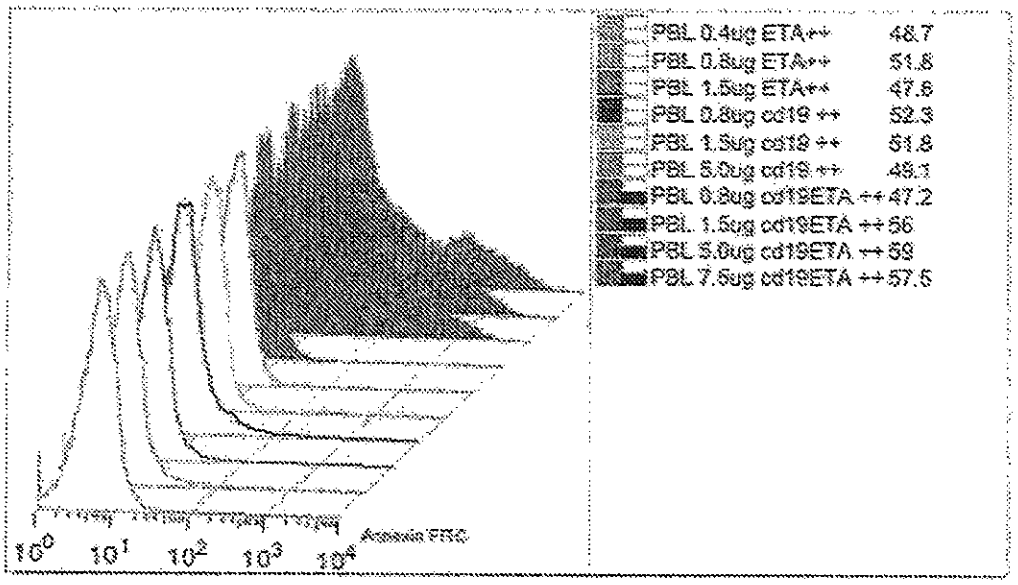

CD19-exotoxin A PBL cell killing
PBL 0.4ug ETA++ 48.7
PBL 0.8ug ETA++ 51.6
PBL 1.5ug ETA++ 47.6
PBL 0.8ug cd19 ++ 52.3
PBL 1.5ug cd19 ++ 51.8
PBL 5.0ug cd19 ++ 49.1
PBL 0.8ug cd19ETA ++ 47.2
PBL 1.5ug cd19ETA ++ 56
PBL 5.0ug cd19ETA ++ 59
PBL 7.5ug cd19ETA ++ 57.5
Annexin FITC
10^0 10^1 10^2 10^3 10^4

FIG. 9

SAA3-Gelonin Sequence

```
   1 CAT ATG TGG GGT ACA TTC CTT AAA GAA GCT GGT CAA GGT GCT AAA GAC ATG TGG AGA GCT 60
   1 H   K   W   G   T   F   L   K   E   A   G   Q   G   A   K   D   M   W   R   A   20

  61 TAC CAA GAC ATG AAA GAA GCT AAC TAC CGT GGT GCA GAC AAA TAC TTC CAC GCT GGT GGT 120
  21 Y   Q   D   M   K   E   A   N   Y   R   G   A   D   K   Y   F   H   A   R   G   40

 121 AAC TAT GAC GCT GCT CGA CGT GGT CCT GGT GGT GCT TGG GCT GCT AAA GTA ATC ACT AAC 180
  41 N   Y   D   A   A   R   R   G   P   G   G   A   W   A   A   K   V   I   S   N   60

 181 GCT AGA GAA ACT ATT CAA GGT ATC ACA GAC CCT CTT TTT AAA GGT ATG ACA CGT GAC CAA 240
  61 A   R   E   T   I   Q   G   I   T   D   P   L   F   K   G   M   T   R   D   Q   80

 241 GTA CGT GAA GAT TGT AAA GCT GAC CAA TTT GCT AAC GAA TGG GGT CGT AGC GGT AAA GAC 300
  81 V   R   E   D   S   K   A   D   Q   F   A   N   E   W   G   R   S   G   K   D   100

 301 CCT AAC CAC TTC AGA CCT GCT GGT CTT CCT GAC AAA TAC TCA GGT GGT GGT GGT TCA CCA 360   G4S2 Linker
 101 P   N   H   F   R   P   A   G   L   P   D   K   Y   S   G   G   G   G   S   P   120

 361 TGG GAA AAT TTA TAT TTT CAA TCA GGT TTA GAT ACA GTT TCA TTT TCA ACA AAA GGT GCT 420   Tev Cleavage Site
 121 W   E   N   L   Y   F   Q   S   G   L   D   T   V   S   F   S   T   K   G   A   140

 421 ACA TAT ATT ACA TAC GTT AAC TTT TTA AAT GAA TTA CGT GTT AAA TTA AAA CCA GAA GGT 480
 141 T   Y   I   T   Y   V   N   F   L   N   E   L   R   V   K   L   K   P   E   G   160

 481 AAT TCA CAT GGT ATT CCA TTA TTA CGT AAA AAA TGT GAT GAT CCA GGT AAA TGT TTT GTT 540
 161 N   S   H   G   I   P   L   L   R   K   K   C   D   D   P   G   K   C   F   V   180

 541 TTA GTT GCT TTA TCA AAT GAT AAT GGT CAA TTA GCT GAA ATT GCT ATT GAT GTT ACA TCA 600
 181 L   V   A   L   S   N   D   N   G   Q   L   A   E   I   A   I   D   V   T   S   200

 601 GTT TAT GTT GTT GGT TAT CAA GTT CGT AAT CGT TCA TAT TTT TTT AAA GAT GCT CCA GAT 660
 201 V   Y   V   V   G   Y   Q   V   R   N   R   S   Y   F   F   K   D   A   P   D   220

 661 GCT GCT TAT GAA GGT TTA TTT AAA AAT ACA ATT AAA ACA CGT TTA CAT TTT GGT GGT TCA 720
 221 A   A   Y   E   G   L   F   K   N   T   I   K   T   R   L   H   F   G   G   S   240   Gelonin

 721 TAT CCA TCA TTA GAA GGT GAA AAA GCT TAT CGT GAA ACA ACA GAT CTT GGT ATT GAA CCA 780
 241 Y   P   S   L   E   G   E   K   A   Y   R   E   T   T   D   L   G   I   E   P   260

 781 CTT CGT ATC GGC ATC AAA AAA CTT GAC GAA AAC GCG ATC GAC AAC TAC AAA CCA ACA GAA 840
 261 L   R   I   G   I   K   K   L   D   E   N   A   I   D   N   Y   K   P   T   E   280

 841 ATC GCG AGC TCT CTT CTT GTT GTA ATC CAA ATG GTA AGC GAA GCG GCA CGT TTC ACA TTC 900
 281 I   A   S   S   L   L   V   V   I   Q   M   V   S   E   A   A   R   F   T   F   300

 901 ATC GAA AAC CAA ATT CGT AAC AAC TTC CAA CAA CGT ATC CGT CCA GCG AAC AAC ACA ATC 960
 301 I   E   N   Q   I   R   N   N   F   Q   Q   R   I   R   P   A   N   N   T   I   320

 961 TCT CTT GAA AAC AAA TGG GGC AAA CTT AGC TTC CAA ATC CGT ACA AGC GGT GCG AAC GGT 1020
 321 S   L   E   N   K   W   G   K   L   S   F   Q   I   R   T   S   G   A   N   G   340

1021 ATG TTC AGC GAA GCG GTA GAA CTT GAA CGC GCG AAC GGC AAA AAA TAC TAC GTA ACT GCG 1080
 341 M   F   S   E   A   V   E   L   E   R   A   N   G   K   K   Y   Y   V   T   A   360

1081 GTA GAT CAA GTA AAA CCA AAA ATC GCA CTT CTT AAA TTC GTA GAC AAA GAC CCA GAA GGT 1140
 361 V   D   Q   V   K   P   K   I   A   L   L   K   F   V   D   K   D   P   E   G   380

1141 ACC TGT TAC GAT TAT AAA GAT CAC GAT GGT GAT TAC AAA GAT CAC GAT ATT GAT TAT AAA 1200   3x Flag
 381 T   C   Y   D   Y   K   D   H   D   G   D   Y   K   D   H   D   I   D   Y   K   400

1201 GAT GAT GAT GAT AAA 1215
 401 D   D   D   D   K   405
```

FIG. 10

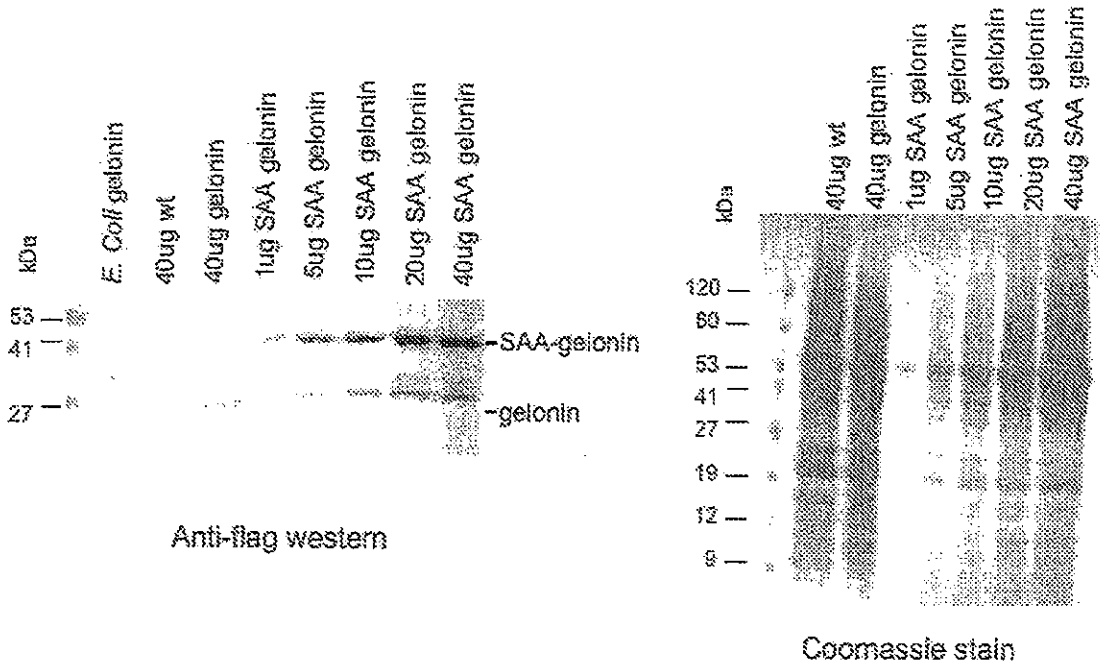

kDa
E. Coli gelonin
40ug wt
40ug gelonin
1ug SAA gelonin
5ug SAA gelonin
10ug SAA gelonin
20ug SAA gelonin
40ug SAA gelonin
53
41
27
SAA-gelonin
-gelonin
Anti-flag western
kDa
40ug wt
40ug gelonin
1ug SAA gelonin
5ug SAA gelonin
10ug SAA gelonin
20ug SAA gelonin
40ug SAA gelonin
120
80
53
41
27
19
12
9
Coomassie stain

FIG. 11

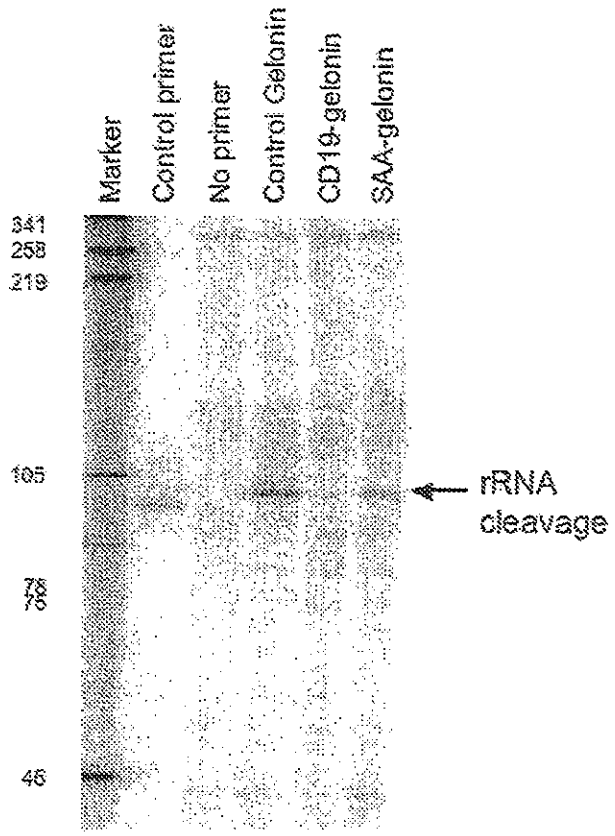

Marker
Control primer
No primer
Control Gelonin
CD19-gelonin
SAA-gelonin
341
258
219
105
78
76
46
rRNA
cleavage

FIG. 12

Gelonin Sequence

```
   1 CAT ATG GGT TTA GAT ACA GTT TCA TTT TCA ACA AAA GGT GCT ACA TAT ATA TAC GTT AAC TTT TTA AAT GAA TTA CGT GTT AAA TTA  90
   1 H   M   G   L   D   T   V   S   F   S   T   K   G   A   T   Y   I   T   Y   V   N   F   L   N   E   L   R   V   K   L  30

  91 AAA CCA GAA GGT AAT TCA CAT GGT ATT CCA TTA TTA CGT AAA AAA TGT GAT GAT CCA GGT AAA TGT TTT GTT TTA GTT GCT TTA TCA AAT 180
  31 K   P   E   G   N   S   H   G   I   P   L   L   R   K   K   C   D   D   P   G   K   C   F   V   L   V   A   L   S   N  60

 181 GAT AAT GGT CAA TTA GCT GAA ATT GCT ATT GAT GTT ACA TCA GTT TAT GTT GTT GGT TAT CAA GTT CGT AAT CGT TCA TAT TTT TTT AAA 270
  61 D   N   G   Q   L   A   E   I   A   I   D   V   T   S   V   Y   V   V   G   Y   Q   V   R   N   R   S   Y   F   F   K  91

 271 GAT GCT CCA GAT gct GCT TAT GAA GGT TTA TTT AAA AAT ACA ATT AAA ACA CGT TTA CAT TTT GGT GGT TCA TAT CCA TCA TTA GAA GGT 360
  90 D   A   P   D   A   A   Y   E   G   L   F   K   N   T   I   K   T   R   L   H   F   G   G   S   Y   P   S   L   E   G  120

 361 GAA AAA GCT TAT CGT GAA ACA ACA GAT CTT GGT ATC GAA CCA CTT CGC ATC GGC ATC AAA AAA CTT GAC GAA AAC GCG ATC GAC AAC TAC 450
 120 E   K   A   Y   R   E   T   T   D   L   G   I   E   P   L   R   I   G   I   K   K   L   D   E   N   A   I   D   N   Y 150

 451 AAA CCA ACA GAA ATC GCG AGC TCT CTT CTT GTT GTA ATC CAA ATG GTA AGC GAA GCG GCA CGT TTC ACA TTC ATC GAA AAC CAA ATT CGT 540
 151 K   P   T   E   I   A   S   S   L   L   V   V   I   Q   M   V   S   E   A   A   R   F   T   F   I   E   N   Q   I   R 180

 541 AAC AAC TTC CAA CAA CGT ATC CGT CCA GCG AAC AAC ACA ATC TCT CTT GAA AAC AAA TGG GGC AAA CTT AGC TTC CAA ATC CGT ACA AGC 630
 181 N   N   F   Q   Q   R   I   R   P   A   N   N   T   I   S   L   E   N   K   W   G   K   L   S   F   Q   I   R   T   S  210

 631 GGT GCG AAC GGT ATG TTC AGC GAA GCG GTA GAA CTT GAA CGC GCG AAC GGC AAA AAA TAC TAC GTA ACT GCG GTA GAT CAA GTA AAA CCA 720
 211 G   A   N   G   M   F   S   E   A   V   E   L   E   R   A   N   G   K   K   Y   Y   V   T   A   V   D   Q   V   K   P  240

 721 AAA ATT GCA CTT CTT AAA TTC GTA GAC AAA GAC CCA GAA GCT ACC TGT TAC GAT TAT AAA GAT GAC GAT GCT GAT TAC AAA GAT GAC GAT 810
 241 K   I   A   L   L   K   F   V   D   K   D   P   E   A   T   C   Y   D   Y   K   D   H   D   G   D   Y   K   D   H   D  270

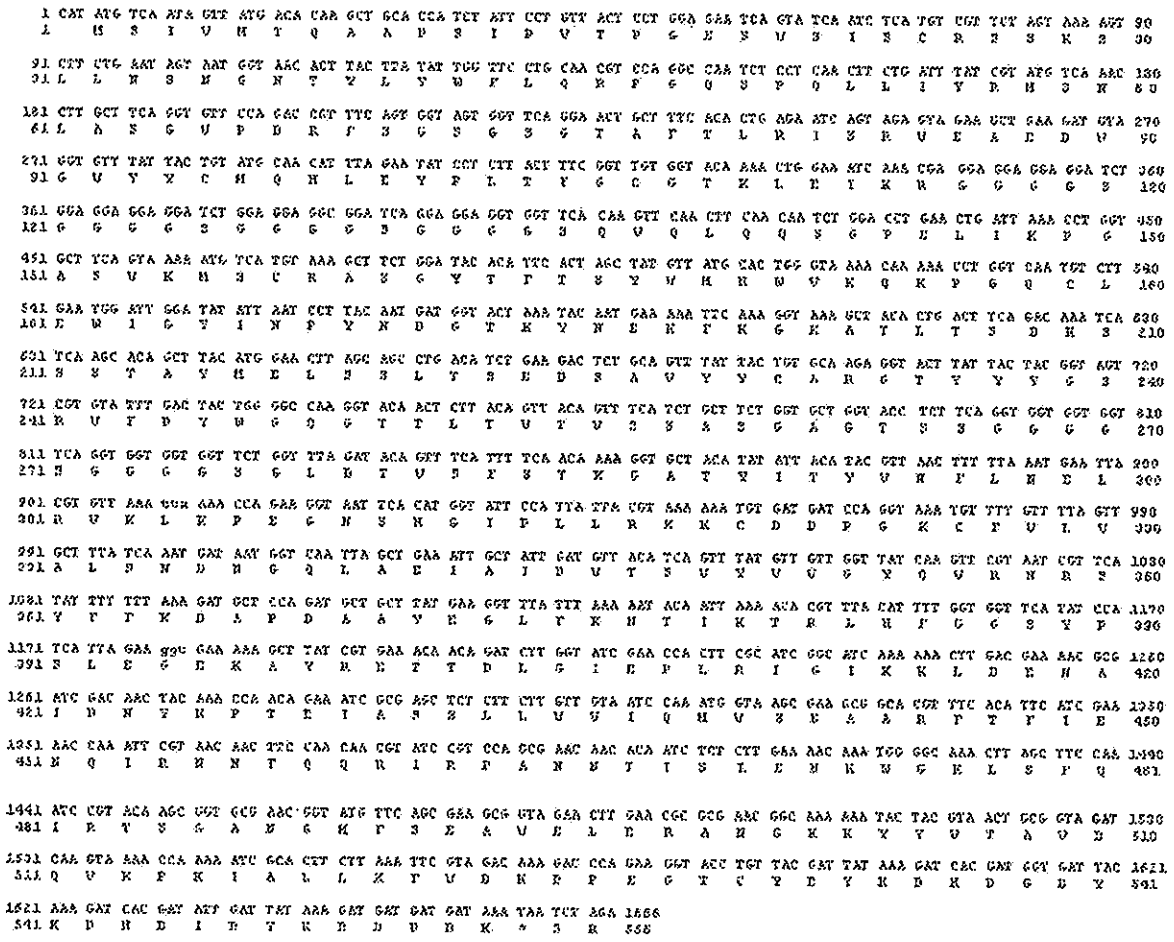
 811 ATT GAT TAT AAA GAT GAT GAT GAT AAA TAA TCT AGA 841
 271 I   D   Y   K   D   D   D   D   K   *   S   R  281
```

Gelonin

3x Flag

FIG. 13

CD19 scFv-Gelonin Sequence



CD19 LC Variable

G_4S Linker

CD19 HC Variable

G_4S Linker

Gelonin

3x Flag

FIG. 14

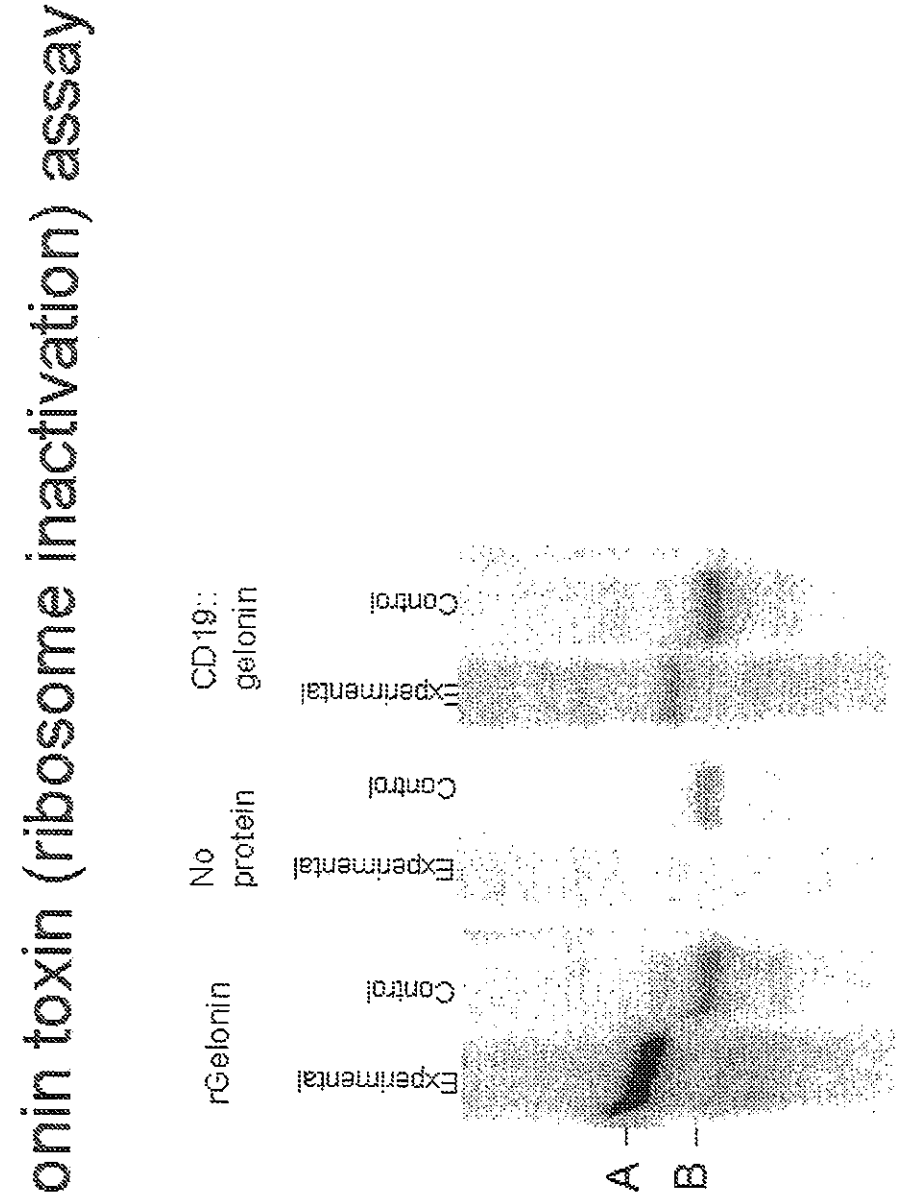

Anti-CD19-gelonin toxin (ribosome inactivation) assay
rGelonin
Experimental
Control
No protein
Experimental
Control
CD19-gelonin
Experimental
Control
A
B

FIG. 15

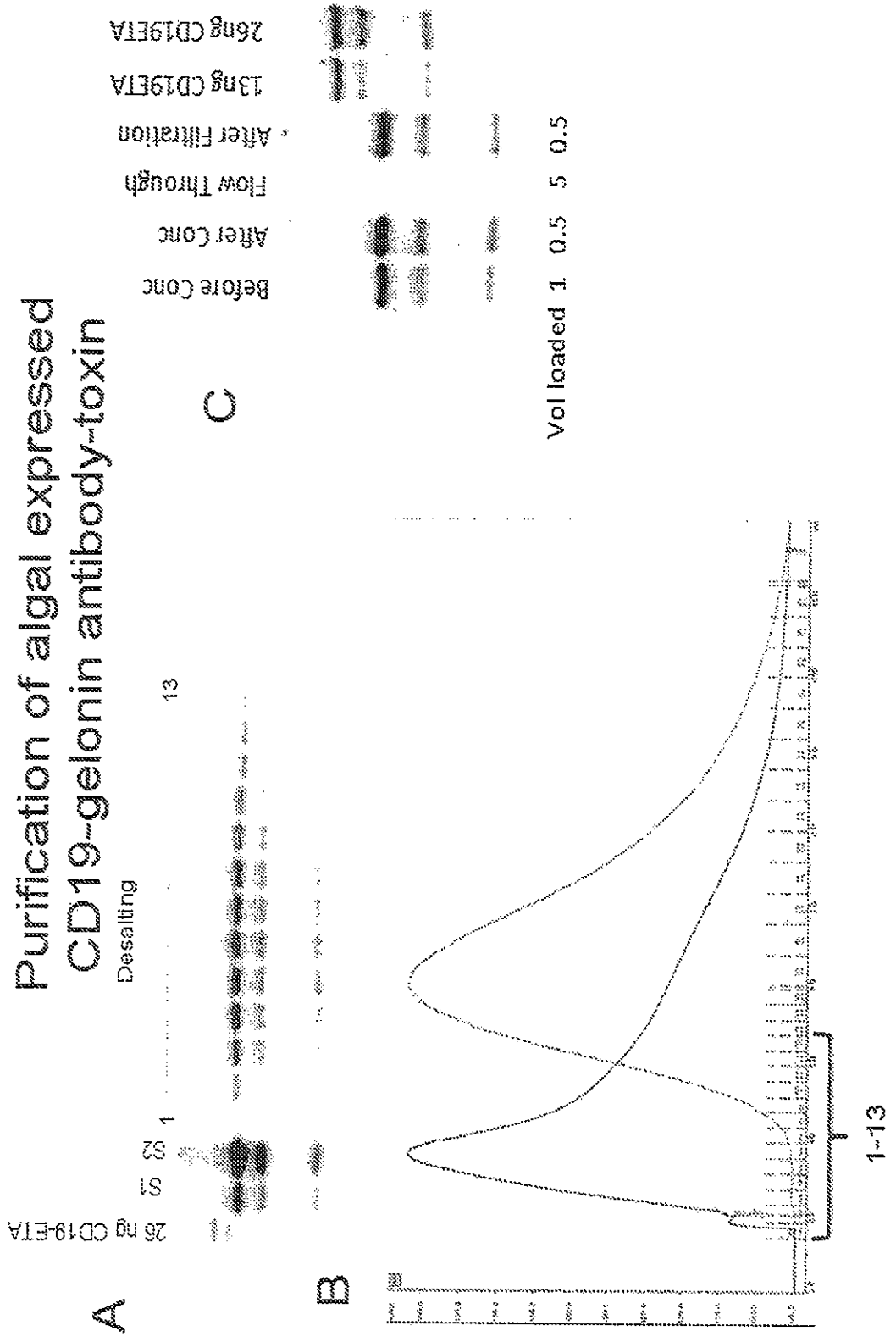

Purification of algal expressed
CD19-gelonin antibody-toxin
A
Desalting
26 ng CD19-ETA
S1
S2
1
13
B
1-13
C
Before Conc
After Conc
Flow Through
After Filtration
13ng CD19ETA
26ng CD19ETA
Vol loaded 1 0.5 5 0.5

FIG. 16

CD19 scFv-CH2-ETA sequence

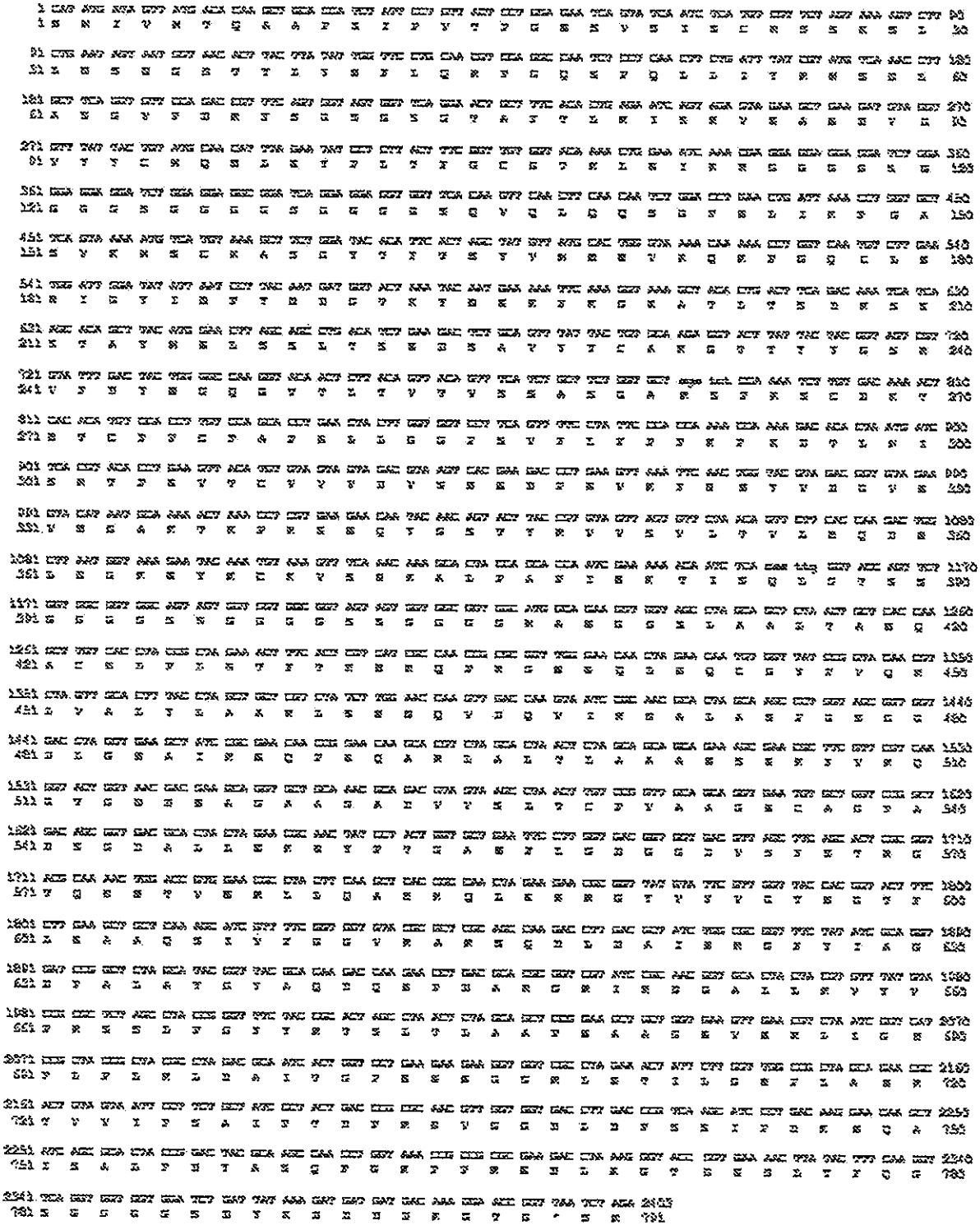


FIG. 17

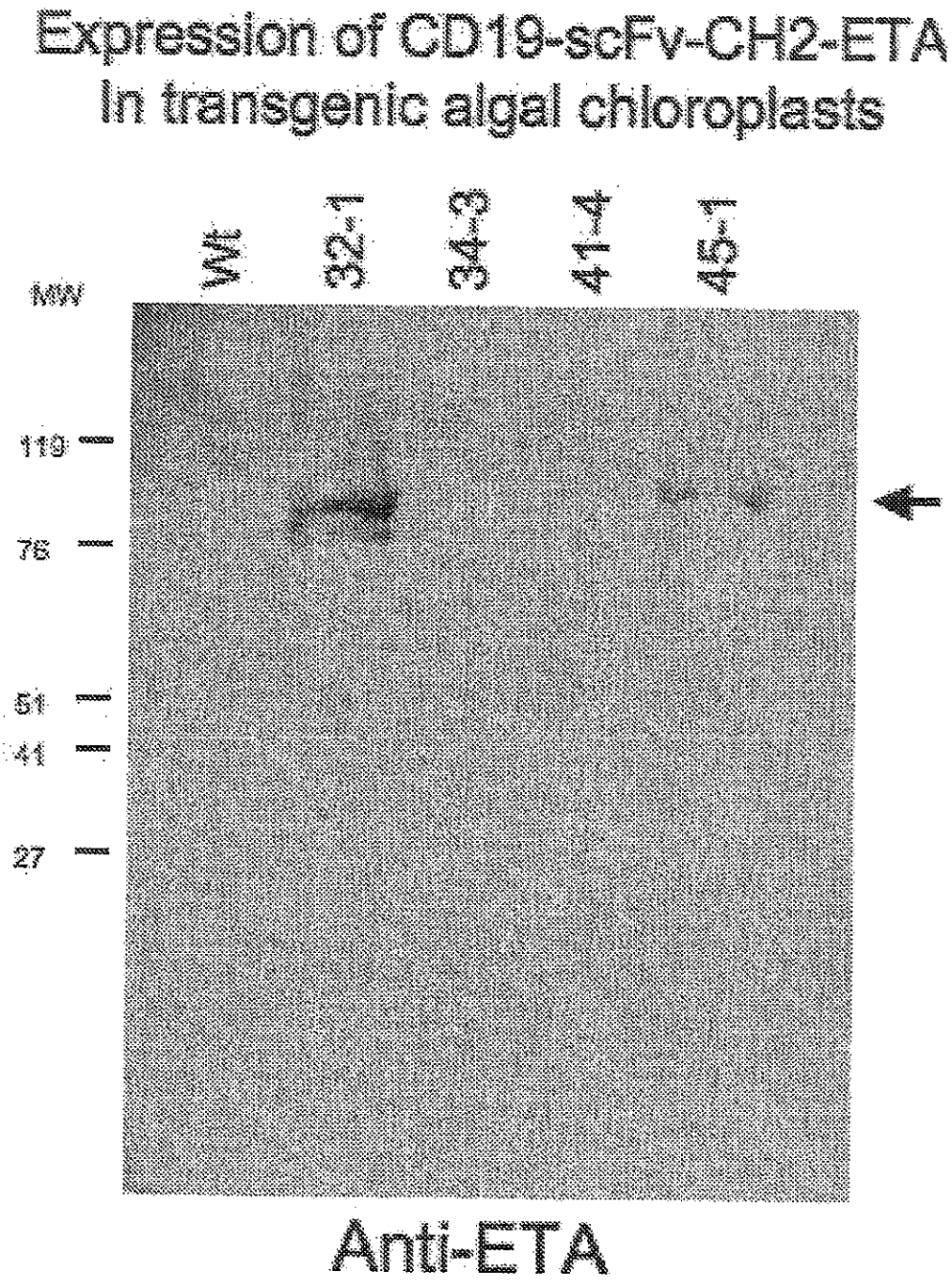

Expression of CD19-scFv-CH2-ETA
In transgenic algal chloroplasts
Wt
32-1
34-3
41-4
45-1
MW
119
76
51
41
27
Anti-ETA

FIG. 18