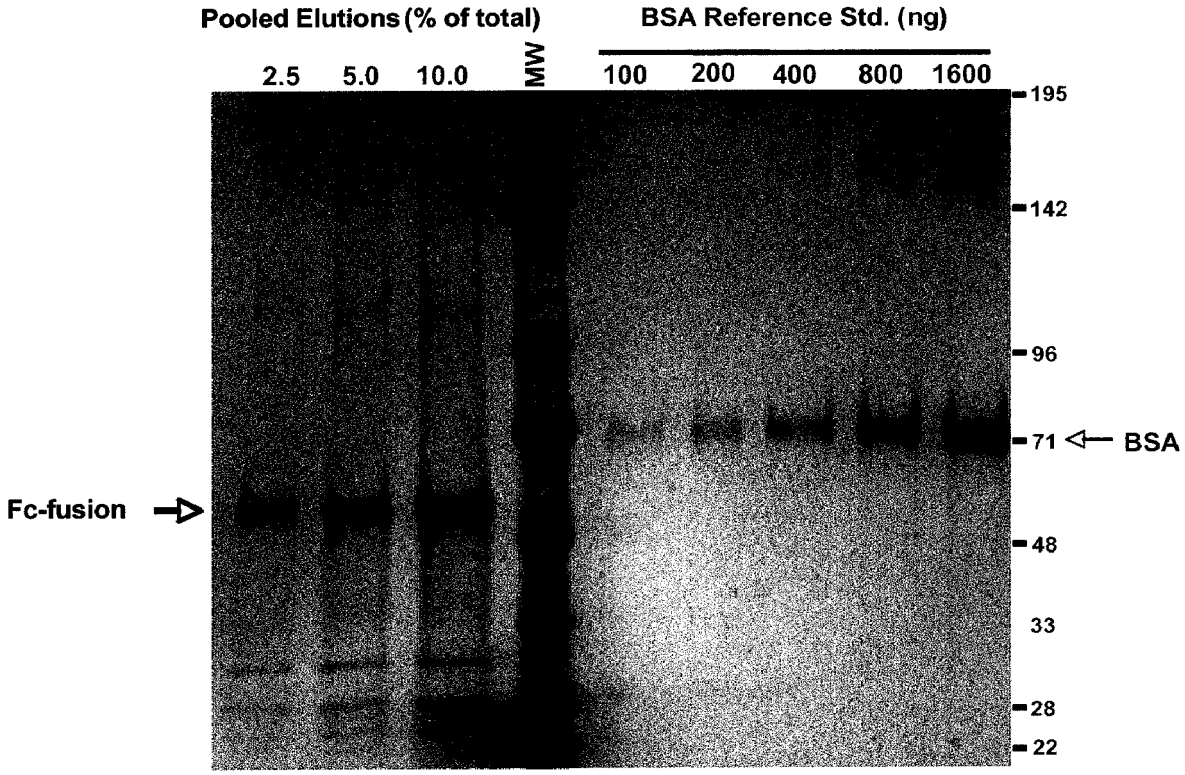

Pooled Elutions (% of total)
BSA Reference Std. (ng)
2.5
5.0
10.0
MW
100
200
400
800
1600
195
142
96
71
BSA
48
33
28
22
Fc-fusion

Figure 1

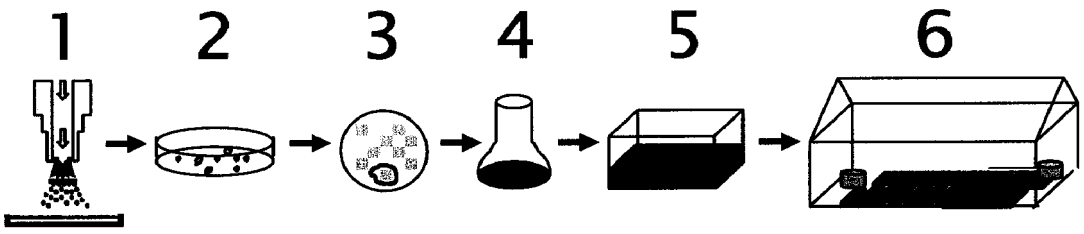


Figure 2

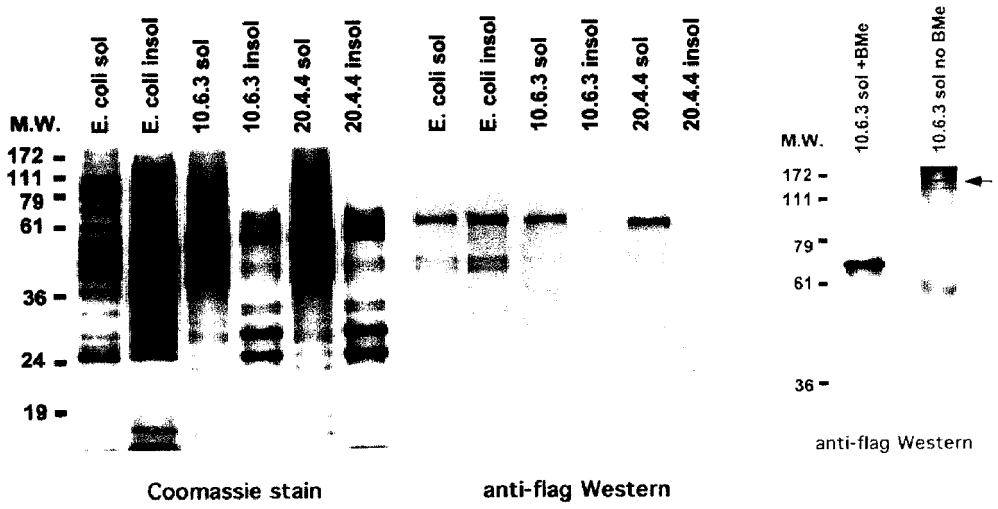

M.W.
E. coli sol
E. coli insol
10.6.3 sol
10.6.3 insol
20.4.4 sol
20.4.4 insol
172
111
79
61
36
24
19
Coomassie stain
anti-flag Western
M.W.
10.6.3 sol +BMe
10.6.3 sol no BMe
anti-flag Western

Figure 3

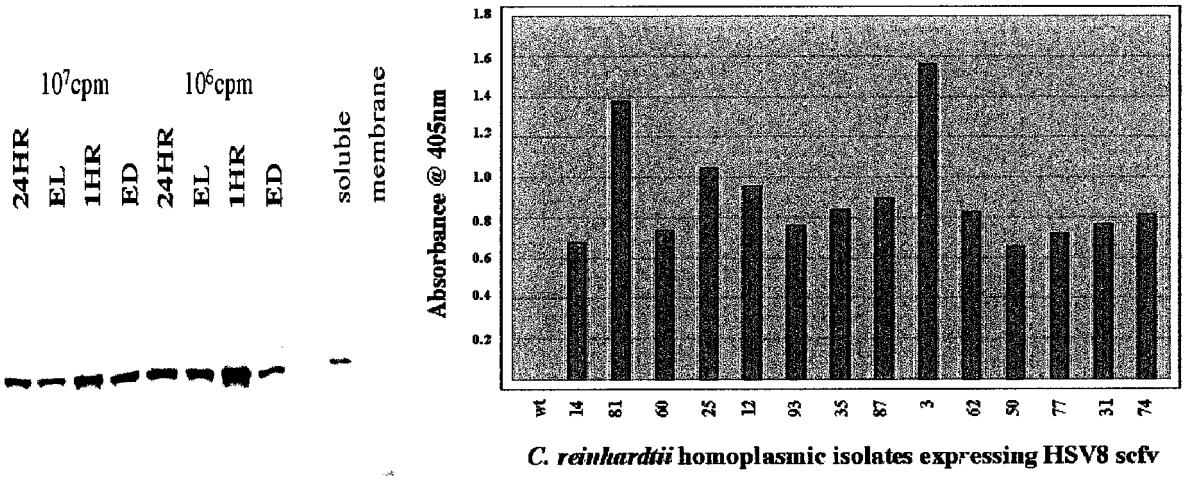

10^7cpm
10^6cpm
24HR
EL
1HR
ED
24HR
EL
1HR
ED
soluble
membrane
Absorbance @ 405nm
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
wt
14
81
60
25
12
93
35
87
3
62
50
77
31
74
C. reinhardtii homoplasmic isolates expressing HSV8 scfv

Figure 4

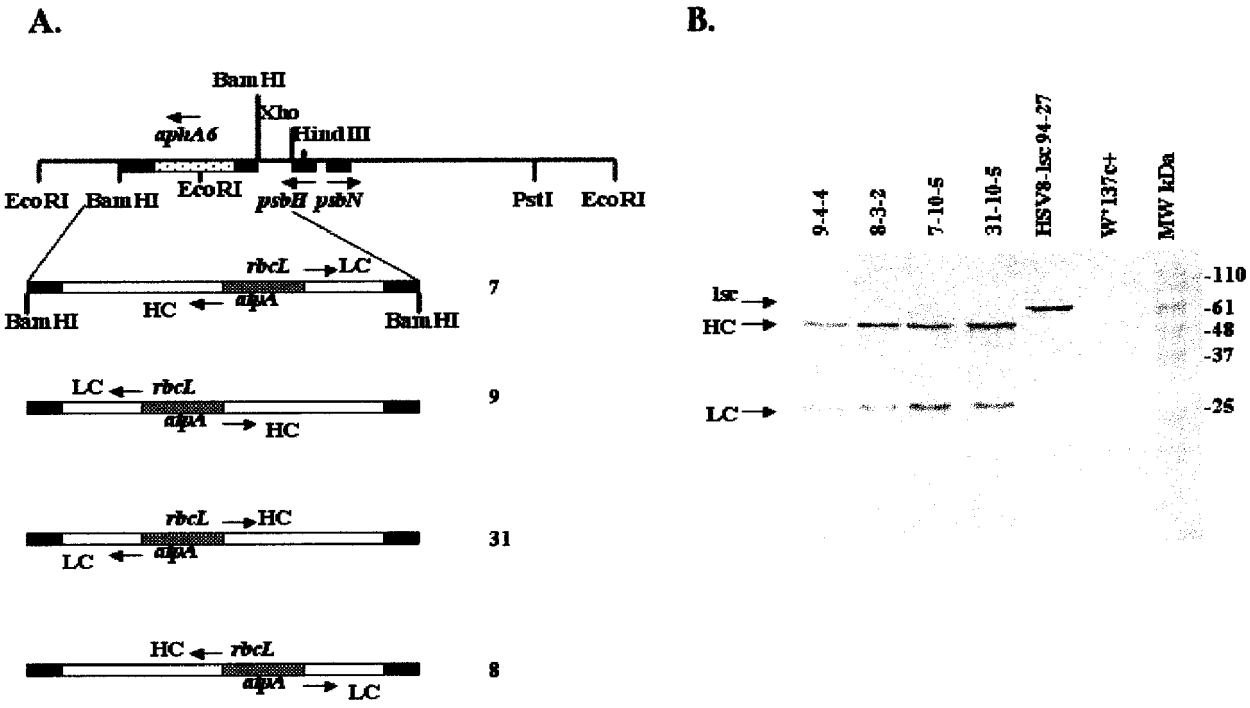

A.
BamHI
XhoI
aphA6
HindIII
EcoRI
BamHI
EcoRI
psbH psbN
PstI
EcoRI
rbcL
LC
HC
atpA
BamHI
BamHI
7
LC
rbcL
atpA
HC
9
rbcL
HC
LC
atpA
31
HC
rbcL
atpA
LC
8
B.
9-4-4
8-3-2
7-10-5
31-10-5
HSV8-lsc 94-27
W^137c+
MW kDa
lsc
HC
LC
-110
-61
-48
-37
-25

Figure 5

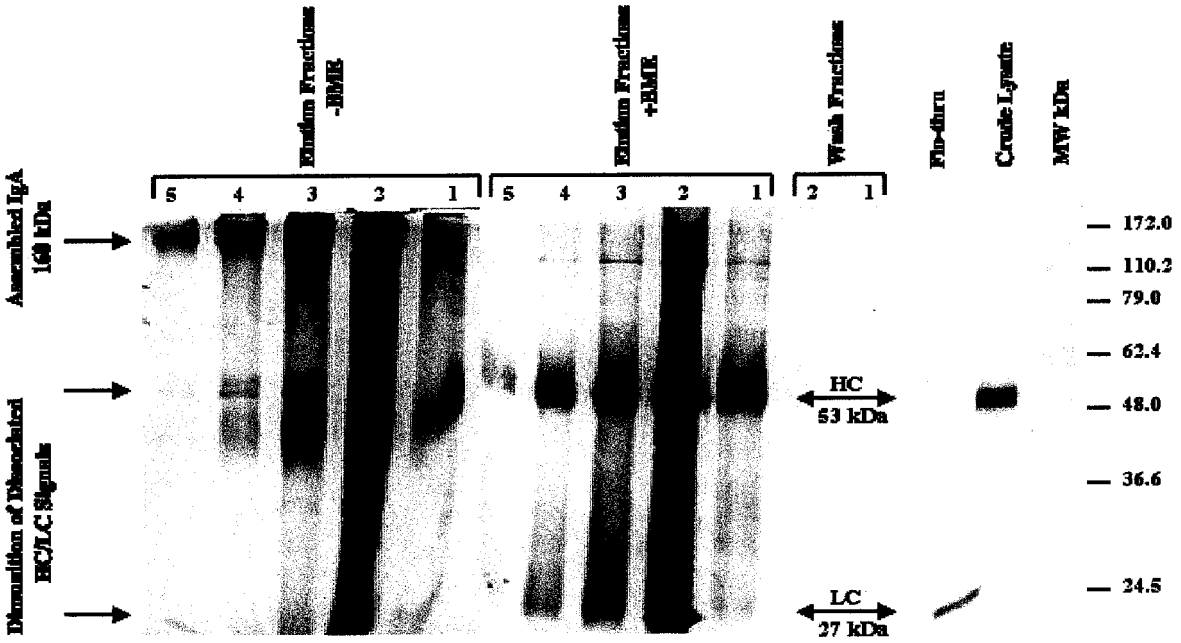

Elution Fractions -BME
Elution Fractions +BME
Wash Fractions
Flo-thru
Crude Lysate
MW kDa
5 4 3 2 1
5 4 3 2 1
2 1
Assembled IgA 160 kDa
Dissolution of Disulfide Bonded HC/LC Signals
HC 53 kDa
LC 27 kDa
172.0
110.2
79.0
62.4
48.0
36.6
24.5

Figure 6

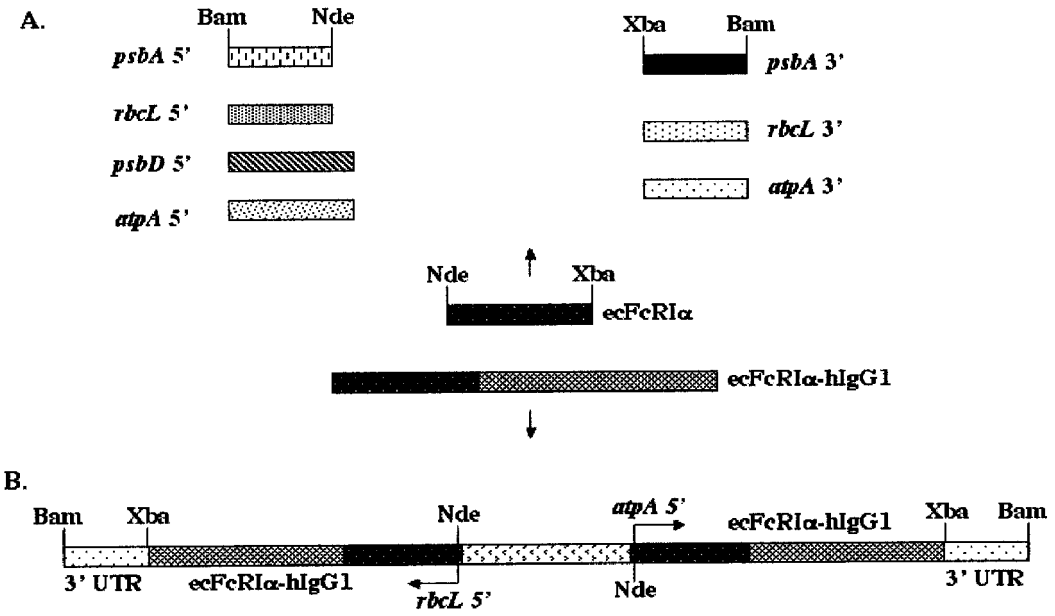

A.
Bam
Nde
psbA 5'
rbcL 5'
psbD 5'
atpA 5'
Xba
Bam
psbA 3'
rbcL 3'
atpA 3'
Nde
Xba
ecFcRIα
ecFcRIα-hIgG1
B.
Bam
Xba
Nde
atpA 5'
ecFcRIα-hIgG1
Xba
Bam
3' UTR
ecFcRIα-hIgG1
rbcL 5'
Nde
3' UTR

Figure 7

RINCON Fc-FUSION APPLICATION

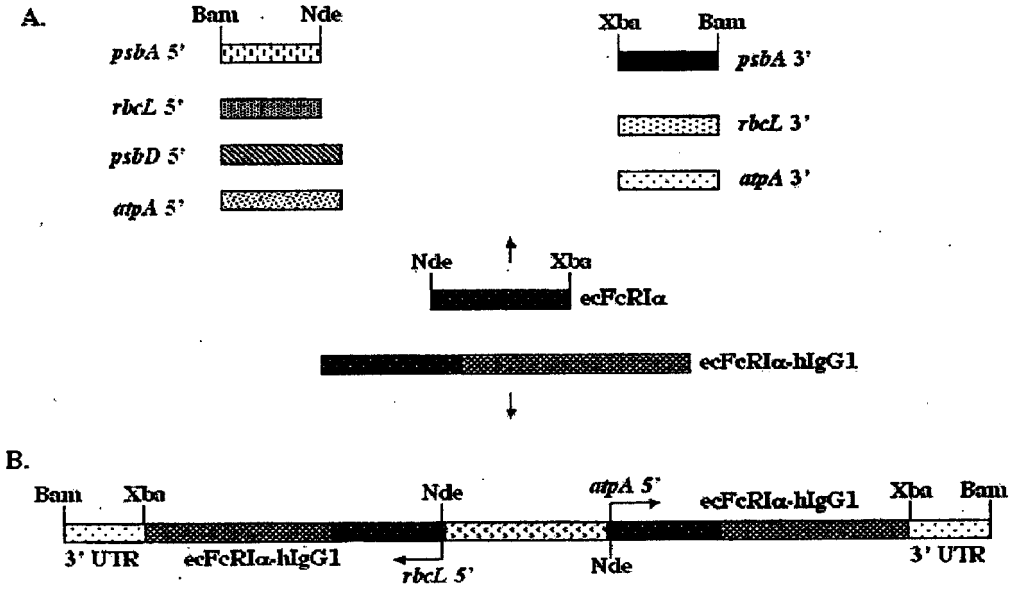
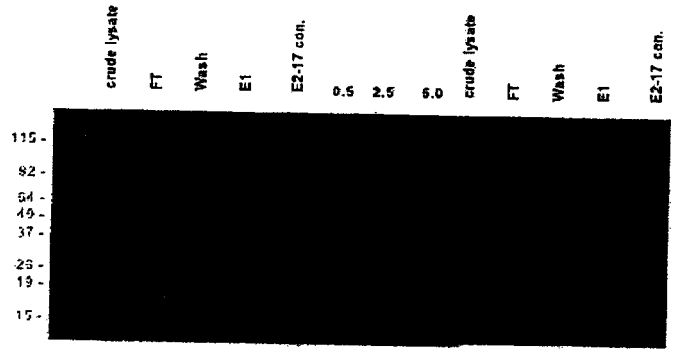


Figure 8



Anti-FLAG Western blot analysis of samples prepared from psbD line expressing the IgERI-Fc receptor fusion. The transplastomic line was grown at two light fluxes, 6 or 58 uEinsteins, as indicated, to a density of $5E^6$ cells per ml. 20 ug of total soluble protein was run in crude lysate lane, while 7.5 ul of sample was run in the Flow thru (FT), 15 ul in wash fractions and 3.75 ul in E1 fractions. The final concentrated sample (E2-17) represents 25% of the final pooled sample. The full length IgERI-Fc receptor fusion has a theoretical MW of 48.7 kDa. FLAG tagged bacterial alkaline phosphatase (BAP FLAG) was run as an internal control.

Figure 9

A. murine light chain gene

ATGGATTACAAAGACCATGATGGTGACTACAAAGATCATGACATTGATTA
TAAAGATGATGATGATAAAGATGTTGTTATGACACAAACACCATTATCAT
TACCAGTTTCATTAGGTGATCAAGCATCAATTTCATGTCGTTCATCACAA
TCATTAGTTCATTCAAATGGTAATACATATTTACATTGGTATTTACAAAA
ACCAGGTCAATCACCAAAATTATTAATTTATAAAGTTTCAAATCGTTTTT
CTGGTGTACCAGATCGTTTTTCTGGTTCTGGTTCTGGTACAGATTTTACA
TTAAAAATTTCTCGTGTAGAAGCTGAAGATTTAGGTGTATATTTTTGTTC
TCAATCTACACATGTACCTTGGACTTTAGGTGGTGGTACCAAATTAGAAA
TTAAACGTGCTGACGCTGCTCCTACTGTATCTATTTTTCCTCCTAGTTCA
GAACAATTAACAAGTGGTGGTGCTAGTGTTGTTTGTTTTTTAAATAATTT
CTATCCAAAAGATATTAATGTTAAATGGAAAATTGATGGTTCAGAACGTC
AAAATGGTGTTTTAAATTCATGGACAGATCAAGATTCAAAAGATTCAACA
TACTCAATGTCATCAACATTAACATTAACTAAAGATGAATACGAACGTCA
TAATTCATACACTTGTGAAGCAACTCATAAAACTTCAACATCACCAATTG
TTAAATCATTTAATCGTAATGAATGTTAA

B. murine kappa light chain protein

MDYKDHDGDYKDHDIDYKDDDDKDVVMTQTPLSLPVSLGDQASISCRSSQS
LVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTL
KISRVEAEDLGVYFCSQSTHVPWTLGGGTKLEIKRADAAPTVSIFPPSSE
QLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTY
SMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

C. murine heavy chain gene

ATGGAAGTTAAATTAGTTGAATCAGGTGGTGGTTTAGTTAAACCAGGAGG
TTCATTAAAATTATCTTGTGCTGCTTCTGGTTTTACATTTTCTAGTTATG
CTATGTCATGGGTTCGTCAAACTCCTGAAAAACGTTTAGAATGGGTAGCA
TCAATTTCATCAGGTGGTTCAACTTATTATCCTGATTCTGTAAAAGGTCG
TTTCACTATTTTCCGTGATAATGCACGTAATATTTTATACTTACAAATGT
CTTCTTTACGTTCAGAAGATACAGCTATGTACTATTGTGTACGTGGTCAA
GACTATTGGGGTCAAGGTACATCAGTAACAGTATCATCAGCTAAAACAAC
ACCACCATCAGTTTATCCATTAGCTCCAGGTTCTGCTGCTCAAACTAATT
CTATGGTTACTTTAGGTTGTTTAGTTAAAGGTTATTTTCCTGAACCTGTA
ACTGTAACTTGGAATAGTGGTTCTTTATCTTCTGGTGTACATACTTTTCC
TGCTGTATTACAATCTGATTTATATACTTTATCTTCTTCAGTAACAGTAC
CATCATCAACATGGCCATCAGAAACAGTAACATGTAATGTAGCACATCCA
GCATCATCAACAAAAGTAGATAAAAAAATTGTTCCAAGAGATTGTGGTTG
TAAACCATGTATTTGTACAGTTCCAGAAGTTTCATCAGTTTTTATTTTCC
CACCAAAACCTAAAGATGTTTTAACAATTACATTAACACCTAAAGTTACA
TGTGTTGTAGTAGATATTTCAAAAGATGATCCTGAAGTTCAATTCTCATG
GTTTGTTGATGATGTTGAAGTTCACACAGCACAAACACAACCAAGAGAAG
AACAATTTAATTCAACATTTAGATCAGTTTCAGAATTACCAATTATGCAT
CAAGATTGGTTAAATGGTAAAGAATTTAAATGTAGAGTTAATTCAGCAGC
ATTCCCAGCACCAATTGAAAAAACAATTTCAAAAACAAAAGGAAGACCAA
AAGCTCCACAAGTTTATACAATTCCACCTCCAAAAGAACAAATGGCTAAA

Figure 10

GATAAAGTTTCATTAACATGTATGATTACAGATTTCTTCCCAGAAGATAT
TACAGTTGAATGGCAATGGAATGGTCAACCAGCTGAAAATTATAAAAATA
CTCAACCTATTATGGATACTGATGGTTCATATTTTGTTTATTCAAAATTA
AATGTTCAAAAATCTAATTGGGAAGCTGGTAATACTTTTACATGTTCTGT
ATTACATGAAGGTTTACACAATCATCATACAGAAAAAATTTTATCTCATT
CTCCAGGTAAAGACTATAAAGATCACGATGGTGATTATAAAGAACACGAT
ATTGATTACAAAGATGATGATGATAAATAA

D. murine heavy chain protein

MEVKLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVA
SISSGGSTYYPDSVKGRFTIFRDNARNILYLQMSSLRSEDTAMYYCVRGQ
DYWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPV
TVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP
ASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVT
CVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMH
QDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAK
DKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKL
NVQKSNWEAGNTFTCSVLHEGLHNHHTEKILSHSPGK***DYKDHDGDYKEHD***
IDYKDDDDK

Figure 10. Nucleotide and amino acid sequences (A and B respectively) for the murine light chain encoding NT73. The FLAG Tag, in B, is indicated by bold italics. Nucleotide and amino acid sequence (C and D respectively) for the murine heavy chain encoding NT73. The FLAG Tag in D is indicated by bold italics.

Figure 10 (Cont.)

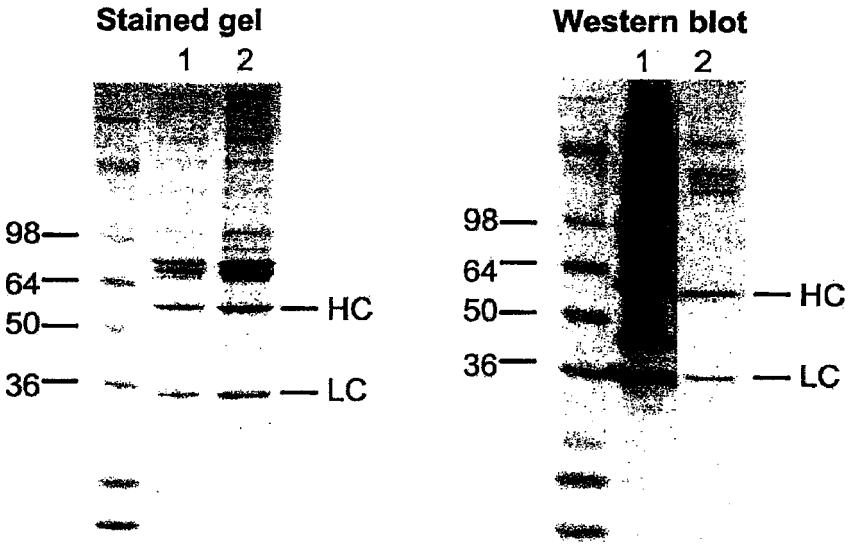


Figure 11. Purification of mIgG1 from C. reinhardtii chloroplasts. Alga expressed mIgG1, purified as described above, were electrophoresed on 4-20% Tris Glycine gels and either stained with Coomassie Brilliant Blue (left panel) or subjected to western blot analysis using anti-FLAG antibody (right panel). Samples run on Coomassie stained gel represent 5 or 10% of the total concentrated lysates

A. chimeric human kappa light chain gene

ATGGATTACAAAGACCATGATGGTGACTACAAAGATCATGACATTGATTA
TAAAGATGATGATGATAAAGATGTAGTAATGACACAAACACCATTATCAT
TACCAGTTTCATTAGGTGATCAAGCTTCAATTTCATGTCGTTCAAGTCAA
AGTTTAGTTCATAGTAATGGTAATACTTATTTACATTGGTATTTACAAAA
ACCAGGTCAATCTCCTAAATTATTAATTTATAAAGTTTCTAACCGTTTTT
CTGGTGTTCCTGACCGTTTTTCTGGATCTGGTTCTGGTACTGATTTCACT
TTAAAAATTTCACGTGTTGAAGCTGAAGATTTAGGAGTATACTTTTGTTC
ACAATCAACACACGTTCCTTGGACATTAGGTGGTGGTACAAAATTAGAAA
TTAAACGTACGGTTGCTGCTCCATCAGTTTTTATTTTTCCACCATCAGAT
GAACAATTAAAATCAGGTACTGCTTCAGTTGTTTGTTTATTAAATAATTT
TTATCCACGTGAAGCTAAAGTTCAATGGAAAGTTGATAATGCTTTACAAT
CAGGTAATTCACAAGAATCAGTTACTGAACAAGATTCAAAAGATTCAACT
TATTCATTATCATCAACTTTAACTTTATCAAAAGCTGATTATGAAAAACA
TAAAGTTTATGCTTGTGAAGTTACTCATCAAGGTTTATCATCACCAGTTA
CTAAATCATTTAATCGTGGTGAATGTTAATCTAGA

B. chimeric human kappa light chain protein

MDYKDHDGDYKDHDIDYKDDDDKDVVMTQTPLSLPVSLGDQASISCRSSQ
SLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFT
LKISRVEAEDLGVYFCSQSTHVPWTLGGGTKLEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

C. chimeric human heavy chain gene

ATGGAAGTTAAATTAGTTGAATCAGGTGGTGGTTTAGTTAAACCAGGAGG
TTCATTAAAATTATCTTGTGCTGCTTCTGGTTTTACATTTTCTTCTTATG
CTATGTCATGGGTTCGTCAAACTCCTGAAAAACGTTTAGAATGGGTAGCA
TCAATTTCATCAGGTGGTTCAACTTATTATCCTGATTCTGTAAAAGGTCG
TTTCACTATTTTCCGTGATAATGCACGTAATATTTTATACTTACAAATGT
CTTCTTTACGTTCAGAAGATACAGCTATGTACTATTGTGTACGTGGTCAA
GACTATTGGGGTCAAGGTACATCAGTAACAGTATCAGCTAGCACAAAAGG
TCCATCTGTTTTCCCATTAGCACCATCATCAAAAAGTACATCTGGTGGTA
CAGCTGCATTAGGTTGTTTAGTTAAAGACTACTTCCCAGAACCTGTAACT
GTATCTTGGAACTCAGGTGCATTAACAAGTGGTGTACACACATTCCCTGC
TGTTTTACAATCATCAGGTTTATACTCATTAAGTAGTGTAGTAACAGTAC
CATCAAGTAGTTTAGGTACACAAACATACATCTGTAACGTAAATCACAAA
CCAAGTAACACAAAAGTAGACAAAAAAGTTGAACCAAAATCTTGTGATAA
AACACACACTTGTCCTCCATGTCCTGCTCCTGAATTATTAGGAGGACCAT
CAGTATTTTTATTCCCACCAAAACCTAAAGATACATTAATGATTTCACGT
ACACCTGAAGTTACTTGTGTAGTAGTAGATGTTTCACATGAAGATCCAGA
AGTTAAATTTAATTGGTATGTTGATGGAGTAGAAGTTCACAATGCAAAAA
CTAAACCACGTGAAGAACAATATAATTCTACATATCGTGTAGTTTCAGTT
TTAACTGTTTTACACCAAGATTGGTTAAATGGTAAAGAATATAAATGTAA
AGTATCAAATAAAGCATTACCTGCACCAATTGAAAAAACTATTTCAAAAG

Figure 12

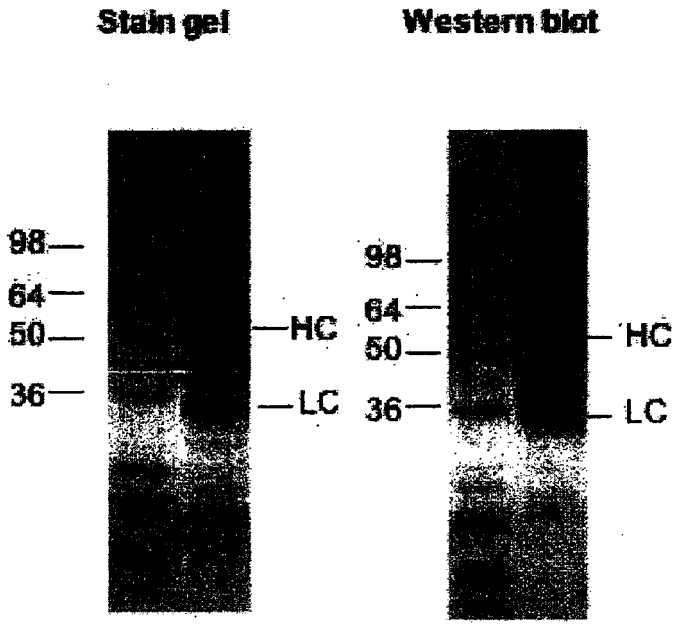
CAAAAGGACAACCTCGTGAACCACAAGTTTATACATTACCACCATCTCGT
GATGAATTAACAAAAAATCAAGTATCATTAACTTGCTTAGTAAAAGGTTT
CTATCCATCAGATATTGCTGTTGAATGGGAATCAAATGGTCAACCAGAAA
ATAATTATAAAACAACTCCTCCAGTTTTAGATTCAGATGGATCATTCTTC
TTATATTCAAAATTAACAGTAGATAAATCTCGTTGGCAACAAGGAAATGT
TTTCTCATGTTCTGTAATGCACGAAGCTTTACACAATCACTATACACAAA
AATCTTTATCTTTATCACCTGGTAAAGACTATAAAGATCACGATGGTGAT
TATAAAGAACACGATATTGATTACAAAGATGATGATGATAAATAA

D. chimeric human heavy chain protein

MEVKLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVA
SISSGGSTYYPDSVKGRFTIFRDNARNILYLQMSSLRSEDTAMYYCVRGQ
DYWGQGTSVTVSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT
VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK
PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK***DYKDHDGD***
YKEHDIDYKDDDDK

Figure 12. Nucleotide and amino acid sequences (A and B respectively) for the chimeric human light chain encoding NT73. The FLAG Tag in B, is indicated by bold italics. Nucleotide and amino acid sequence (C and D respectively) for the chimeric human heavy chain encoding NT73. The FLAG Tag in D is indicated by bold italics.

Figure 12 (Cont.)



Purification of hIgG1 from C. reinhardtii chloroplasts. Alga expressed hIgG1, purified as described above, were electrophoresed on 4-20% Tris Glycine gels and either stained with Coomassie Brilliant Blue (left panel) or subjected to western blot analysis using anti-FLAG antibody (right panel). Samples run on Coomassie stained gel represent 5 % of the total concentrated lysates.

Figure 13