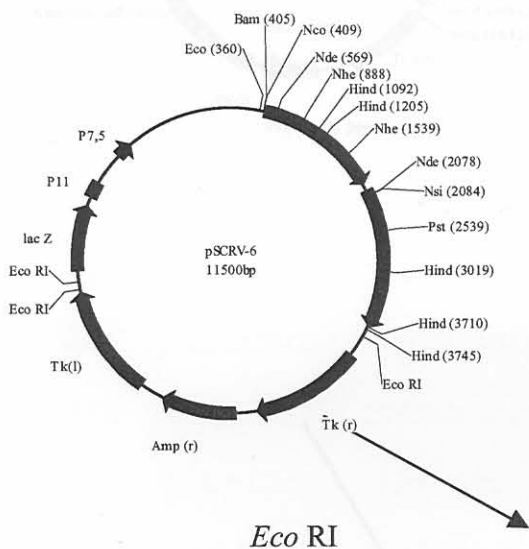
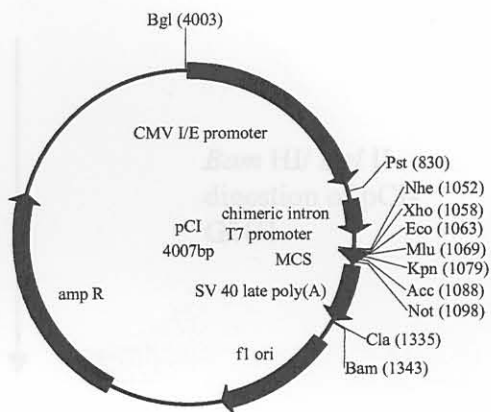


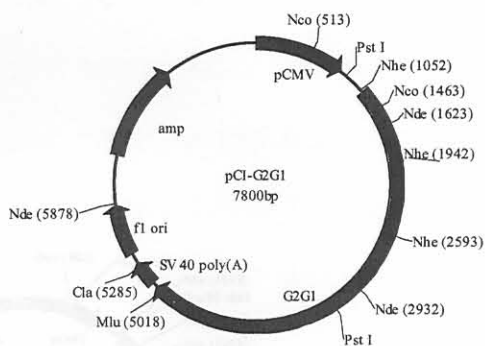
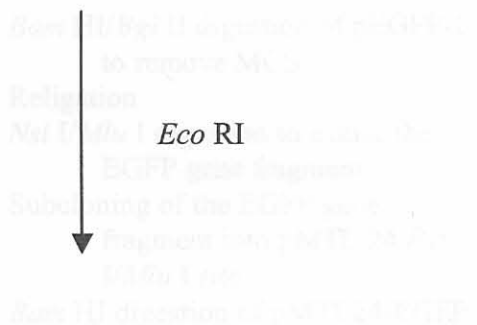
ADDENDUM I

Eco RI digestion of the pCI mammalian expression vector.

Eco RI excision of the 3.8 kb G2G1 gene fragment from the pSCRV-6 construct.

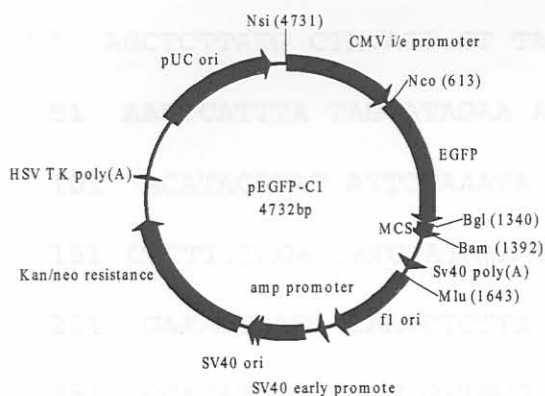


Eco RI



APPENDIX II

pMTL24-EGFP-1.0
 pMTL24-EGFP-1.0: r1ft2
 check: 475 from: 1 to: 710
 r1ft2_9.seq
 Length: 710



Bam HI/ *Bgl* II digestion of pCI-G2G1.

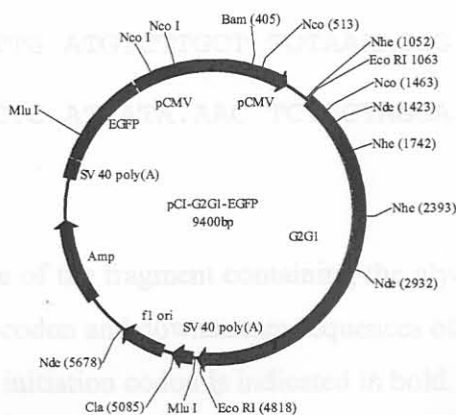
Bam HI/*Bgl* II digestion of pEGFP-C1 to remove MCS.

Religation

Nsi I/*Mlu* I digestion to excise the EGFP gene fragment

Subcloning of the EGFP gene fragment into pMTL 24 *Pst* I/*Mlu* I site

Bam HI digestion of pMTL24-EGFP



ADDENDUM II

DNA_SEQUENCE 1.0

FROMSTADEN of: rift2

check: 875 from: 1 to: 710

<rift2.00001----->

rift2_9.seq

Length: 710

1 AGCTCTTATG CTA.AGT.CT TAATACGACT CACTATA.GC TAGCCTCGAG

51 **AATTCATTTA** TAGCATAGAA AAAAACAAAA TGAAATTCTA CTATATTTTT

101 ACATACATAT ATTCTAAATA TGAAAGTGGT GATTGTGACT AGCGTAGCAT

151 CGCTT.TAGA CATCTATATA CTATATAGTA ATACCAATAC TCAAGACTAC

201 GAAACTGATA CAATCTCTTA TCATGTGGGT AATGTTCTCG ATGTCGATAG

251 CCATATGCCC GGT.GTTGCG ATATACATAA ACTGATCACT AATTCCAAAC

301 CCACCCGCTT TTTATAGTAA GTTTTTTACC CATAAATAAT AAATACAATA

351 ATTAATTTCT CGTAAAAGTA GAAAATATAT TCTAATTTAT TGCACGGTAA

401 GGAAGTAGAA TCATAAAGAA CAG.GACGGA TCCCC**ATGG** CAGGGATTGC

451 AATGACAGTC CTTCCAGCCT TA.CAGTTTT TGCTTTGGCA CCTGTTGTTT

501 TTGCTGAAGA CCCCATCTC AGAAACAGAC CAGGGAAGGG GCACAACACTAC

551 ATTGAC.GGA TGA²CTCATGA GGATGCCACA TGCAAACCTG TGACATATGC

601 TGGGGCATGT AGCAGTTTTG ATGTCTTGCT TGTA²AAAGGG AAAATTTCCC

651 C.TTTTCCAG .CGTATGCTC AT.ATA.AAC TCTACTAGGA GGCAGGTTTA

701 CGACACCATT

DNA sequence of the insertion site of the fragment containing the glycoprotein genes of the RVFV as well as the initiation codon and downstream sequences of the G2 gene. The *Eco* RI insertion site as well as the initiation codon is indicated in bold. The insertion site was confirmed to be correct.

ADDENDUM III

>ref|NC_002044.1| Rift Valley fever virus M segment, complete sequence
 Length = 3884

Score = 400 bits (202), Expect = e-109
 Identities = 264/274 (96%), Gaps = 8/274 (2%)
 Strand = Plus / Plus

```

429 ccatggcagggattgcaatgacagtccttccagcctta-cagtttttgctttggcacctg 487
    |||||||
409 ccatggcagggattgcaatgacagtccttccagccttagcagtttttgctttggcacctg 468

488 ttgtttttgctgaagacccccatctcagaaacagaccaggggaaggggcacaactacattg 547
    |||||||
469 ttgtttttgctgaagacccccatctcagaaacagaccaggggaaggggcacaactacattg 528

548 ac-ggatgactcatgaggatgccacatgcaaacctgtgacatatgctggggcatgtagca 606
    |||||||
529 acgggatgactcaggaggatgccacatgcaaacctgtgacatatgctggggcatgtagca 588

607 gttttgatgtcttgcttgtaaaagggaaaatttccccttttccag-cgtatgctcat-at 664
    |||||||
589 gttttgatgtcttgcttg-aaaagggaaaatttccccttttccagtcgtatgctcatcat 647

665 a-aactctactaggaggcaggtttacgacaccat 697
    |
648 agaactctacta-gaggca-gttcacgacaccat 679
  
```

Alignment of the sequences obtained with the RVFV confirming the presence of the RVFV glycoprotein genes in the fragment cloned from nucleotide 429, representing the initiation codon starting position 411 in the M segment. The initiation codon was found to be intact and in frame.

ADDENDUM IV

RVFV specific ELISA titres as OD values of a 1:10 dilution of mouse sera after vaccination of mice with a DNA construct containing the glycoprotein genes.

Pre-boost (week 3)		Pre-challenge (week 9)	
Sample	OD	Sample	OD
pCI 1	-0,082	pCI 1	-0,04
pCI 2	-0,114	pCI 2	0,033
pCI-G2G1-EGFP 1	-0,061	pCI-G2G1-EGFP 1	0,007
pCI-G2G1-EGFP 2	-0,047	pCI-G2G1-EGFP 2	0,012
pCI-G2G1-EGFP 3	0,043	pCI-G2G1-EGFP 3	0,014
		+ ve 1	-0,024
		+ ve 2	-0,078
		- ve 1	-0,012
		- ve 2	0,006
OD = (OD pos Ag) – (OD Neg Ag)			

ADDENDUM V

Temperature values taken daily for one week after challenge of the experimental animals with a virulent field strain of Rift Valley fever virus.

Days post challenge

DNA i.m.	1	2	3	4	5	6	7	8	9
3283	40.5	41.4	39.2	39	39.2	39.8	39.2	39.4	38.9
3345	39.8	41.6	39.6	40.2	39.8	39.4	39.4	39	39.4
3287	40	40.7	39.5	39.3	38.9	39.2	39.1	39.2	39
Avg	40.1	41.2	39.4	39.5	39.3	39.5	39.2	39.2	39.1
Sd	0.36	0.47	0.21	0.62	0.46	0.31	0.15	0.20	0.26
DNA with Quil A	1	2	3	4	5	6	7	8	9
3383	40.3	40.2	39.3	39.9	39.1	39.5	39.5	40	39.4
3368	40	39.7	39.4	41.3	39.4	39.5	39.4	39.3	39.1
3372	40.6	42	39.5	39.3	39.8	38.9	39.4	39.5	39
Avg	40.3	40.6	39.4	40.2	39.4	39.3	39.4	39.6	39.2
Sd	0.30	1.21	0.10	1.03	0.35	0.35	0.06	0.36	0.21
DNA i.d.	1	2	3	4	5	6	7	8	9
3313	39.3	39.8	39.7	39.2	38.7	39.2	39.6	39	39.5
3347	39.5	41.1	40	39.3	39.3	39.2	41.2	39.5	39
3373	40.3	40.6	40.1	41.5	39.4	40.1	39.6	39.5	39.5
Avg	39.7	40.5	39.9	40.0	39.1	39.5	40.1	39.3	39.3
Sd	0.53	0.66	0.21	1.30	0.38	0.52	0.92	0.29	0.29
Negative controls	1	2	3	4	5	6	7	8	9
3004-Control	41.5	41.2	39.9	39.8	39.6	39.8	39.5	39.9	39.3
3670-Control	40.7	40.6	39.9	39.3	39.4	39.4	39.3	39.4	39.6
Avg	41.1	40.9	39.9	39.55	39.5	39.6	39.4	39.65	39.45
Sd	0.57	0.42	0.00	0.35	0.14	0.28	0.14	0.35	0.21

ADDENDUM VI

Liver enzyme levels of trial animals after inoculation with the pCI-G2G1-EGFP DNA vaccine construct and challenge with a virulent Rift Valley fever virus strain.

DNA i.m.	Basal			Day 2			Day 3			Day 6		
	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH
3283	47	51	19	58	40	7	70	69	51	52	37	12
3345	59	57	22	89	54	25	120	55	65	110	55	52
3287	44	60	40	45	54	7	56	39	24	40	51	3
Avg	50	56	27	64	49	13	82	54	47	67	48	22

DNA with Quil A	Basal			Day 2			Day 3			Day 6		
	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH
3383	53	40	3	50	48	7	52	41	5	49	48	3
3368	46	64	34	40	51	27	31	45	8	179	72	271
3372	60	67	28	50	47	5	60	34	5	69	48	19
Avg	53	57	22	47	49	13	48	40	6	99	56	98

DNA i.d.	Basal			Day 2			Day 3			Day 6		
	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH
3347	75	63	28	79	47	20	146	47	91	183	78	67
3373	48	68	5	72	59	11	79	49	13	145	59	21
3313	60	84	30	50	67	7	49	56	4	88	66	23
Avg	61	72	21	67	58	13	91	51	36	139	68	37

Nega tive g	Basal			Day 2			Day 3			Day 6		
	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH
3010	51	49	35	52	39	10	52	37	13	58	45	29
3670	42	62	17	48	54	9	47	41	15	68	53	13
Avg	47	56	26	50	47	10	50	39	14	63	49	21

Positive	Basal			Day 2			Day 3			Day 6		
	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH	AST	GGT	GLDH
3643	182	147	255	130	117	63	148	123	67	258	218	149
3648	66	69	47	51	59	9	54	42	11	67	63	12
3650	52	63	34	45	58	3	39	50	2	40	58	12
Avg	100	93	112	75	78	25	80	71	26	121	113	57

1. Bredaar, T.G., Blackburn, N.K. (1997). An immunised Rift Valley fever vaccine. *Journal of the South African Veterinary Association* 48(1): 45-48.
2. Bagarozzi, M.L., Boyer, J.D., Upton, K.L., Jeradisa, M.A., Chatterjee, M., Shah, A., Bennett, A., Ciccardi, R., Carrara, R., Casey, L., Weiner, D.J. (1988). Safety and immunogenicity of HIV-1 DNA constructs in chimpanzees. *Faculty* 16(10): 116-124.
3. Boyer, J.D., Jeradisa, M.A. (1997). Biological safety of genetic immunisation. *Journal of Virology* 71(1): 7-8-12.
4. Barry, M.A., Fox, M.C., Johnston, S.A. (1995). Protection against a lymphoma infection using recombinant DNA immunisation. *Nature* 377: 61, 67.
5. Bredaar, T.G., Wessels, E.W. (1998). Out on the town with DNA-vaccines. *Current Biology* 16: 1315-1318.
6. Bredaar, T.G., Blackburn, N.K. (1991). Topological mapping of antigenic sites on the Rift Valley fever virus envelope glycoprotein using monoclonal antibodies. *Trends in virology* 12(1): 111-124.
7. Bredaar, T.G., Blackburn, N.K. (1992). The synergistic neutralisation of Rift Valley fever virus by monoclonal antibodies to the envelope glycoprotein. *Journal of virology* 123: 259-259.
8. Buhner, D.H.L., Callaway, C.H., Condit, J., Chudakov, M.P., Gaidamovich, S.Ya., Hannon, C., Ivics, D.E., Marshall, J.D., Oistr-Mann, N., Peterson, R.F., Porterfield, J.S., Rosati, P.K., Shope, R.E., Wetstap, E.G. (1980). *Herpesviridae*. *Inter-virology* 14: 125-143.
9. Bystic, C.M., Moris, M., Wetstap, E.G., Robinson, H.L. (1996). Role of different lymphoid tissues in the initiation and maintenance of DNA-based antibody responses to the influenza virus H1 glycoprotein. *Journal of Virology*, 20(12): 9074-9078.