

SEQUENCE LISTING

<110> Sementis Limited

<120> VIRAL VECTOR MANUFACTURE

<130> 35226179/WJP

<160> 11

<170> PatentIn version 3.5

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<211> 2031

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<223> Nucleotide sequence of CP77 codon optimised for expression in mammalian cells (CHO)

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Glu Val Val Lys Leu Leu Leu Asp Ser Gly Thr Asn Pro Leu His Lys
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Asn Trp Arg Gln Leu Thr Pro Leu Gly Glu Tyr Thr Asn Ser Arg His
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Gly Lys Val Asn Lys Asp Ile Ala Met Val Leu Leu Glu Ala Thr Gly
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Tyr Ser Asn Ile Asn Asp Phe Asn Ile Phe Thr Tyr Met Lys Ser Lys
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Asn Val Asp Ile Asp Leu Ile Lys Val Leu Val Glu His Gly Phe Asp
115 120 125

Phe Ser Val Lys Cys Glu Lys His His Ser Val Ile Glu Asn Tyr Val
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tattattagt atgtaagtgt aaatataata aaacttaata tctattcaaa ttaataaata	11640
aacctcgata tacagaccga taaaacacat gcgtcaattt tacgcatgat tatctttaac	11700
gtacgtcaca atatgattat ctttctaggg ttaagaagac tg	11742

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 <211> 54
 <212> DNA
 <213> Artificial

<220>
 <223> Forward primer for CP77-CHO gene from pPH51 plasmid

<400> 4
 aacacgtctc ggggggcccgc caccatgttc gactacctgg aaaatgagga agtg 54

<210> 5
 <211> 65
 <212> DNA
 <213> Artificial

<220>
 <223> Reverse primer for CP77-CHO gene from pPH51 plasmid

<400> 5
 caggaagacg ctttttcact tgtcatcgtc atccttgtaa tcctgctgct cgaagatctt 60
 gtact 65

<210> 6
 <211> 855
 <212> DNA
 <213> Artificial

<220>
 <223> Nucleotide sequence of vaccinia Copenhagen K1L gene (extract from Genbank M35027.1)

<400> 6
 atggatctgt cacgaattaa tacttggaag tctaagcagc tgaaaagctt tctctctagt 60
 aaagatacat ttaaggcgga tgtccatgga catagtgcct tgtattatgc aatagctgat 120
 aataacgtgc gtctagtatg tacgttggtg aacgctggag cattgaaaaa tcttctagag 180
 aatgaatttc cattacatca ggcagccaca ttagaagata ccaaaatagt aaagattttg 240
 ctattcagtg gaatggatga ttcacaattt gatgacaaag gaaacaccgc attgtattat 300
 gcggttgata gtggtaacat gcaaacggtg aaactgtttg ttaagaaaaa ttggagactg 360
 atgttctatg ggaaaactgg atggaaaact tcattttatc atgccgtcat gcttaatgat 420
 gtaagtattg tatcatactt tctttcagaa ataccatcta cttttgatct ggctattctc 480
 cttagtgtga ttcacaccac tataaaaaat ggacacgtgg atatgatgat tctcttgctc 540
 gactatatga cgtcgacaaa caccaataat tcccttctct tcattccgga cattaaattg 600
 gctatagata ataaagacat tgagatgtta caggctctgt tcaaatacga cattaatattc 660
 tactctgtta atctggaaaa tgtactattg gatgatgccg aaataactaa gatgattata 720
 gaaaagcatg ttgaatacaa gtctgactcc tatacaaaag atctcgatat cgtcaagaat 780
 aataaattgg atgaaataat tagcaaaaac aaggaactca gactcatgta cgtcaattgt 840
 gtaaagaaaa actaa 855

<210> 7
 <211> 284
 <212> PRT
 <213> Artificial

<220>

<223> Amino acid sequence of vaccinia Copenhagen K1L (extract from Genbank M35027.1) encoded by SEQ ID NO: 6

<400> 7

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Phe Leu Ser Ser Lys Asp Thr Phe Lys Ala Asp Val His Gly His Ser
20 25 30

Ala Leu Tyr Tyr Ala Ile Ala Asp Asn Asn Val Arg Leu Val Cys Thr
35 40 45

Leu Leu Asn Ala Gly Ala Leu Lys Asn Leu Leu Glu Asn Glu Phe Pro
50 55 60

Leu His Gln Ala Ala Thr Leu Glu Asp Thr Lys Ile Val Lys Ile Leu
65 70 75 80

Leu Phe Ser Gly Met Asp Asp Ser Gln Phe Asp Asp Lys Gly Asn Thr
85 90 95

Ala Leu Tyr Tyr Ala Val Asp Ser Gly Asn Met Gln Thr Val Lys Leu
100 105 110

Phe Val Lys Lys Asn Trp Arg Leu Met Phe Tyr Gly Lys Thr Gly Trp
115 120 125

Lys Thr Ser Phe Tyr His Ala Val Met Leu Asn Asp Val Ser Ile Val
130 135 140

Ser Tyr Phe Leu Ser Glu Ile Pro Ser Thr Phe Asp Leu Ala Ile Leu
145 150 155 160

Leu Ser Cys Ile His Thr Thr Ile Lys Asn Gly His Val Asp Met Met
165 170 175

Ile Leu Leu Leu Asp Tyr Met Thr Ser Thr Asn Thr Asn Asn Ser Leu
180 185 190

Leu Phe Ile Pro Asp Ile Lys Leu Ala Ile Asp Asn Lys Asp Ile Glu
195 200 205

Met Leu Gln Ala Leu Phe Lys Tyr Asp Ile Asn Ile Tyr Ser Val Asn
210 215 220

Leu Glu Asn Val Leu Leu Asp Asp Ala Glu Ile Thr Lys Met Ile Ile
225 230 235 240

Glu Lys His Val Glu Tyr Lys Ser Asp Ser Tyr Thr Lys Asp Leu Asp
245 250 255

Ile Val Lys Asn Asn Lys Leu Asp Glu Ile Ile Ser Lys Asn Lys Glu
260 265 270

Leu Arg Leu Met Tyr Val Asn Cys Val Lys Lys Asn
275 280

<210> 8
<211> 551
<212> PRT
<213> Artificial

<220>
<223> VACV-COP-D13L ORF

<400> 8

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Lys Arg Ser Asn Val Phe Ala Val Asp Ser Gln Ile Pro Thr Leu Tyr
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Met Pro Gln Tyr Ile Ser Leu Ser Gly Val Met Thr Asn Asp Gly Pro
35 40 45

Asp Asn Gln Ala Ile Ala Ser Phe Glu Ile Arg Asp Gln Tyr Ile Thr
50 55 60

Ala Leu Asn His Leu Val Leu Ser Leu Glu Leu Pro Glu Val Lys Gly
65 70 75 80

Met Gly Arg Phe Gly Tyr Val Pro Tyr Val Gly Tyr Lys Cys Ile Asn
85 90 95

His Val Ser Ile Ser Ser Cys Asn Gly Val Ile Trp Glu Ile Glu Gly
100 105 110

Glu Glu Leu Tyr Asn Asn Cys Ile Asn Asn Thr Ile Ala Leu Lys His
115 120 125

Ser Gly Tyr Ser Ser Glu Leu Asn Asp Ile Ser Ile Gly Leu Thr Pro
130 135 140

Asn Asp Thr Ile Lys Glu Pro Ser Thr Val Tyr Val Tyr Ile Lys Thr
145 150 155 160

Pro Phe Asp Val Glu Asp Thr Phe Ser Ser Leu Lys Leu Ser Asp Ser
165 170 175

Lys Ile Thr Val Thr Val Thr Phe Asn Pro Val Ser Asp Ile Val Ile
180 185 190

Arg Asp Ser Ser Phe Asp Phe Glu Thr Phe Asn Lys Glu Phe Val Tyr
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195

200

205

Val Pro Glu Leu Ser Phe Ile Gly Tyr Met Val Lys Asn Val Gln Ile
 210 215 220

Lys Pro Ser Phe Ile Glu Lys Pro Arg Arg Val Ile Gly Gln Ile Asn
 225 230 235 240

Gln Pro Thr Ala Thr Val Thr Glu Val His Ala Ala Thr Ser Leu Ser
 245 250 255

Val Tyr Thr Lys Pro Tyr Tyr Gly Asn Thr Asp Asn Lys Phe Ile Ser
 260 265 270

Tyr Pro Gly Tyr Ser Gln Asp Glu Lys Asp Tyr Ile Asp Ala Tyr Val
 275 280 285

Ser Arg Leu Leu Asp Asp Leu Val Ile Val Ser Asp Gly Pro Pro Thr
 290 295 300

Gly Tyr Pro Glu Ser Ala Glu Ile Val Glu Val Pro Glu Asp Gly Ile
 305 310 315 320

Val Ser Ile Gln Asp Ala Asp Val Tyr Val Lys Ile Asp Asn Val Pro
 325 330 335

Asp Asn Met Ser Val Tyr Leu His Thr Asn Leu Leu Met Phe Gly Thr
 340 345 350

Arg Lys Asn Ser Phe Ile Tyr Asn Ile Ser Lys Lys Phe Ser Ala Ile
 355 360 365

Thr Gly Thr Tyr Ser Asp Ala Thr Lys Arg Thr Ile Phe Ala His Ile
 370 375 380

Ser His Ser Ile Asn Ile Ile Asp Thr Ser Ile Pro Val Ser Leu Trp
 385 390 395 400

Thr Ser Gln Arg Asn Val Tyr Asn Gly Asp Asn Arg Ser Ala Glu Ser
 405 410 415

Lys Ala Lys Asp Leu Phe Ile Asn Asp Pro Phe Ile Lys Gly Ile Asp
 420 425 430

Phe Lys Asn Lys Thr Asp Ile Ile Ser Arg Leu Glu Val Arg Phe Gly
 435 440 445

Asn Asp Val Leu Tyr Ser Glu Asn Gly Pro Ile Ser Arg Ile Tyr Asn
 450 455 460

Glu Leu Leu Thr Lys Ser Asn Asn Gly Thr Arg Thr Leu Thr Phe Asn
 Page 14

465 470 475 480

Phe Thr Pro Lys Ile Phe Phe Arg Pro Thr Thr Ile Thr Ala Asn Val
485 490 495

Ser Arg Gly Lys Asp Lys Leu Ser Val Arg Val Val Tyr Ser Thr Met
500 505 510

Asp Val Asn His Pro Ile Tyr Tyr Val Gln Lys Gln Leu Val Val Val
515 520 525

Cys Asn Asp Leu Tyr Lys Val Ser Tyr Asp Gln Gly Val Ser Ile Thr
530 535 540

Lys Ile Met Gly Asp Asn Asn
545 550

<210> 9
<211> 1680
<212> DNA
<213> Artificial

<220>
<223> CHO codon optimised nucleotide sequence encoding SEQ ID NO:1

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ggcgtgatga ccaacgacgg ccctgacaac caggctatcg cctccttcga gatccgggat 180
cagtacatca ccgccctgaa ccacctggtg ctgtccctgg aactgcccga agtgaagggc 240
atgggcagat tcggctacgt gccctacgtg ggctacaagt gcatcaacca cgtgtccatc 300
tccagctgca acggcgtgat ctgggaaatc gagggcgagg aactgtacaa caactgcatt 360
aacaacacaa tcgccctgaa gcaactccggc tactcctccg agctgaacga catctccatc 420
ggcctgaccc ccaacgacac catcaaagaa ccctccaccg tgtacgtgta catcaagacc 480
cccttcgacg tggaagatac cttctccagc ctgaagctgt ccgactccaa gatcaccgtg 540
accgtgacct tcaaccctgt gtccgacatc gtgatccggg actccagctt cgacttcgag 600
acattcaaca aagaatttgt gtacgtgccc gagctgtcct tcacggtgta catggtcaag 660
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cagcctaccg ccaccgtgac agaggtgcac gccgccacat ccctgagcgt gtacaccaag 780
ccctactacg gcaacaccga caacaagttc atctcctacc ccggctacag ccaggacgag 840
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ttcgcccaca tctcccacag catcaacatc atcgacacct ccatccccgt gtccctgtgg	1200
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cggatctaca acgagctgct gaccaagtcc aacaacggca ccagaaccct gacctttaac	1440
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gacaagctga gcgtgcgggt ggtgtactcc accatggacg tgaaccaccc catctactac	1560
gtgcagaaac agctgggtgt cgtgtgcaac gatctgtaca aggtgtccta cgaccagggc	1620
gtgtccatta ccaagatcat gggcgataac aacgactaca aggacgacga cgacaagtga	1680

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 <212> DNA
 <213> synthetic primer

<400> 10	
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<210> 11
 <211> 40
 <212> DNA
 <213> synthetic primer

<400> 11	
caggaagacg ctttttact tgtcgtcgtc gtcctttag	40

<210> 12
 <211> 8
 <212> PRT
 <213> Artificial

<220>
 <223> Flag-Tag protein

<400> 12

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 13
 <211> 16
 <212> PRT
 <213> Artificial

<220>
 <223> D13L peptide

<400> 13

Cys Tyr Asp Gln Gly Val Ser Ile Thr Lys Ile Met Gly Asp Asn Asn
1 5 10 15

<210> 14

<211> 35
 <212> DNA
 <213> synthetic primer

 <400> 14
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 <210> 15
 <211> 38
 <212> DNA
 <213> synthetic primer

 <400> 15
 aatttagtgt gcgcgtggaa aaagcttaca ataaactc 38

 <210> 16
 <211> 36
 <212> DNA
 <213> synthetic primer

 <400> 16
 atatttaa atgcgcgaata atggaacaag aaccct 36

 <210> 17
 <211> 36
 <212> DNA
 <213> synthetic primer

 <400> 17
 cgactctaga ggatcgcgct gaggtcggca actacg 36

 <210> 18
 <211> 668
 <212> PRT
 <213> Artificial

 <220>
 <223> CP77 protein comprising sequence of CPXV-125L Brighton Red

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 Asn Asn Ala Leu His Ala Tyr Leu Phe Asn Glu His Cys Asn Asn Val
 35 40 45
 Glu Val Val Lys Leu Leu Leu Asp Ser Gly Thr Asn Pro Leu His Lys
 50 55 60
 Asn Trp Arg Gln Leu Thr Pro Leu Gly Glu Tyr Thr Asn Ser Arg His
 65 70 75 80
 Gly Lys Val Asn Lys Asp Ile Ala Met Val Leu Leu Glu Ala Thr Gly
 85 90 95

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Tyr Ser Asn Ile Asn Asp Phe Asn Ile Phe Thr Tyr Met Lys Ser Lys
 100 105 110
 Asn Val Asp Ile Asp Leu Ile Lys Val Leu Val Glu His Gly Phe Asp
 115 120 125
 Phe Ser Val Lys Cys Glu Lys His His Ser Val Ile Glu Asn Tyr Val
 130 135 140
 Met Thr Asp Asp Pro Val Pro Glu Ile Ile Asp Leu Phe Ile Glu Asn
 145 150 155 160
 Gly Cys Ser Val Ile Tyr Glu Asp Glu Asp Asp Glu Tyr Gly Tyr Ala
 165 170 175
 Tyr Glu Glu Tyr His Ser Gln Asn Asp Asp Tyr Gln Pro Arg Asn Cys
 180 185 190
 Gly Thr Val Leu His Leu Tyr Ile Ile Ser His Leu Tyr Ser Glu Ser
 195 200 205
 Asp Ser Arg Ser Cys Val Asn Pro Glu Val Val Lys Cys Leu Ile Asn
 210 215 220
 His Gly Ile Asn Pro Ser Ser Ile Asp Lys Asn Tyr Cys Thr Ala Leu
 225 230 235 240
 Gln Tyr Tyr Ile Lys Ser Ser His Ile Asp Ile Asp Ile Val Lys Leu
 245 250 255
 Leu Met Lys Gly Ile Asp Asn Thr Ala Tyr Ser Tyr Ile Asp Asp Leu
 260 265 270
 Thr Cys Cys Thr Arg Gly Ile Met Ala Asp Tyr Leu Asn Ser Asp Tyr
 275 280 285
 Arg Tyr Asn Lys Asp Val Asp Leu Asp Leu Val Lys Leu Phe Leu Glu
 290 295 300
 Asn Gly Lys Pro His Gly Ile Met Cys Ser Ile Val Pro Leu Trp Arg
 305 310 315 320
 Asn Asp Lys Glu Thr Ile Ser Leu Ile Leu Lys Thr Met Asn Ser Asp
 325 330 335
 Val Leu Gln His Ile Leu Ile Glu Tyr Ile Thr Phe Ser Asp Ile Asp
 340 345 350
 Ile Ser Leu Val Glu Tyr Met Leu Glu Tyr Gly Ala Val Val Asn Lys
 355 360 365

Glu Ala Ile His Gly Tyr Phe Lys Asn Ile Asn Ile Asp Ser Tyr Thr
 370 375 380
 Met Lys Tyr Leu Leu Lys Lys Glu Gly Gly Asp Ala Val Asn His Leu
 385 390 395 400
 Asp Asp Gly Glu Ile Pro Ile Gly His Leu Cys Lys Ser Asn Tyr Gly
 405 410 415
 Arg Tyr Asn Phe Tyr Thr Asp Thr Tyr Arg Gln Gly Phe Arg Asp Met
 420 425 430
 Ser Tyr Ala Cys Pro Ile Leu Ser Thr Ile Asn Ile Cys Leu Pro Tyr
 435 440 445
 Leu Lys Asp Ile Asn Met Ile Asp Lys Arg Gly Glu Thr Leu Leu His
 450 455 460
 Lys Ala Val Arg Tyr Asn Lys Gln Ser Leu Val Ser Leu Leu Leu Glu
 465 470 475 480
 Ser Gly Ser Asp Val Asn Ile Arg Ser Asn Asn Gly Tyr Thr Cys Ile
 485 490 495
 Ala Ile Ala Ile Asn Glu Ser Arg Asn Ile Glu Leu Leu Asn Met Leu
 500 505
 Leu Cys His Lys Pro Thr Leu Asp Cys Val Ile Asp Ser Leu Arg Glu
 515 520 525
 Ile Ser Asn Ile Val Asp Asn Ala Tyr Ala Ile Lys Gln Cys Ile Arg
 530 535 540
 Tyr Ala Met Ile Ile Asp Asp Cys Ile Ser Ser Lys Ile Pro Glu Ser
 545 550 555 560
 Ile Ser Lys His Tyr Asn Asp Tyr Ile Asp Ile Cys Asn Gln Glu Leu
 565 570 575
 Asn Glu Met Lys Lys Ile Ile Val Gly Gly Asn Thr Met Phe Ser Leu
 580 585 590
 Ile Phe Thr Asp His Gly Ala Lys Ile Ile His Arg Tyr Ala Asn Asn
 595 600 605
 Pro Glu Leu Arg Ala Tyr Tyr Glu Ser Lys Gln Asn Lys Ile Tyr Val
 610 615 620
 Glu Val Tyr Asp Ile Ile Ser Asn Ala Ile Val Lys His Asn Lys Ile
 625 630 635 640

His Lys Asn Ile Glu Ser Val Asp Asp Asn Thr Tyr Ile Ser Asn Leu
645 650 655

Pro Tyr Thr Ile Lys Tyr Lys Ile Phe Glu Gln Gln
660 665

<210> 19
<211> 2903
<212> DNA
<213> Artificial

<220>
<223> CP77+DsRed expression cassette sequence

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cattcacttc tattcagtca aaatgtttga ttatctggaa aatgaggagg tggctctcga 180
tgaacttaaa cagatgttga gagatagaga tcctaataatgat accaggaacc aattcaagaa 240
taatgctcta catgcatacc ttttcaatga gcactgtaaat aatgtttgagg ttgtcaaact 300
actactagac agtgggtacta atccattaca caaaaattgg agacagctta ctccattagg 360
ggaatacaca aatagtagac atggtaaagt taataaggat atagcgatgg ttctactaga 420
agctactgga tatagcaaca taaatgactt taatatattc acctatatga aatccaaaaa 480
tgtagatatt gacttgataa aggtattgggt agaacatgga tttgatttta gtgttaaattg 540
cgaaaaacat cattcagtta tagaaaatta tgtaatgaca gatgatcctg ttcctgaaat 600
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cggatgcg tatgaagaat atcactcaca aaatgacgat tatcaaccac gaaattgcgg 720
tacagtatta catctgtata tcactctc tctgtattca gagtcggatt cgagatcatg 780
tgtgaacccg gaagttgtta aatgtctgat taatcatgga atcaaccat cttctataga 840
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cgtaaattg ttaatgaaag gaatagataa cacggcttat tcatatatag acgatctaac 960
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