

~~SEQUENCE LISTING~~

~~<110> WuXi Biologics (shanghai) Co., Ltd.
WuXi Biologics Ireland Limited~~

~~<120> ENHANCED EXPRESSION SYSTEM AND METHODS OF USE THEREOF~~

~~<130> 6711-20183111B~~

~~<150> 202010102211.1~~

~~<151> 2020-02-19~~

~~<150> PCT/CN2020/081464~~

~~<151> 2020-03-26~~

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<211> 3949

<212> DNA

<213> Artificial Sequence

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<211> 1128

<212> DNA

<213> Artificial Sequence

<400> 39

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<210> 40

<211> 1352

<212> DNA

<213> Artificial Sequence

<400> 40

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<210> 41

<211> 1246

<212> DNA

<213> Artificial Sequence

<400> 41

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<210> 42

<211> 1886

<212> DNA

<213> Artificial Sequence

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<210> 43

<211> 1118

<212> DNA

<213> Artificial Sequence

<400> 43

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tgtagctga gagctctggg ttctgtggagc gactctgggt cagtgaataa gatagagagt	180
gacatcagct ttgggcttcc acagcaaatg agctcacttg catgcaaca gaaatgcaaa	240
cacatgcaca aagcaaaaca aaaggaacac aggccaaagg tgggtcattc ctataccatc	300
ccctcagcag ggtgcagtcc ccacacctg acccagttcc ctcatgatgt tagagaaaat	360
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~~<210> 44~~

~~<211> 1611~~

~~<212> DNA~~

~~<213> Artificial Sequence~~

~~<400> 44~~

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~~<210> 45~~

~~<211> 1299~~

~~<212> DNA~~

~~<213> Artificial Sequence~~

~~<400> 45~~

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~~ggcgtgcact taccctgctt gttagaaagta aattggcctg tttagccatg caatgctgtt 180~~
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ttataaaattt	at ttttttctg	ctaattctttt	tatttttaaat	tggttcttca	caatcttaag	600
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agtgtcaggt	tgcatgacat	gttttggact	acttgatttt	aggagcctaa	tattccctgg	1260
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<210> 46

<211> 1108

<212> DNA

<213> Artificial Sequence

<400> 46

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gctgagagct	ctgggttcgt	ggagcgactc	tggttcagt g	aataagatag	agagtgcacat	180
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<210> 47

<211> 3619

<212> DNA

<213> Artificial Sequence

<400> 47

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gggactctgt	gctcgtaaaa	cagcacgttg	cttctcatac	caggaccatt	ttgcttcagc	180
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<211> 1114

<212> DNA

<213> Artificial Sequence

<400> 48

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<210> 49

<211> 4068

<212> DNA

<213> Artificial Sequence

<400> 49

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<211> 1128

<212> DNA

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<211> 1352

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<211> 1246

<212> DNA

<213> Artificial Sequence

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<210> 54

<211> 1886

<212> DNA

<213> Artificial Sequence

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<210> 55

<211> 1118

<212> DNA

<213> Artificial Sequence

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<210> 56

<211> 1611

<212> DNA

<213> Artificial Sequence

<400> 56

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<212> DNA

<213> Artificial Sequence

<400> 57

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 35 40 45
 Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser Tyr Arg
 50 55 60
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 65 70 75 80
 Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
 85 90 95
 Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
 100 105 110
 Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
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 180 185 190
 Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
 195 200 205
 Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Thr Pro Arg Asp
 210 215 220
 Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val
 225 230 235 240
 Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr
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 Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu
 260 265 270
 Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln
 275 280 285
 Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser
 290 295 300
 Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys
 305 310 315 320
 Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile
 325 330 335
 Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro
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 Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met
 355 360 365~~

30

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<400> 72

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~~100 105 110~~
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~~115 120 125~~
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~~275 280 285~~

31

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~~Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val~~
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SEQUENCE LISTING

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WuXi Biologics Ireland Limited

<120> ENHANCED EXPRESSION SYSTEM AND METHODS OF USE THEREOF

<130> 6711-2018311IB

<150> 202010102211.1

<151> 2020-02-19

<150> PCT/CN2020/081464

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<213> Artificial Sequence

<220>

<223> Reverse Complement Regulatory Element Sequence 03

<400> 18

tgtgcttaga gacataaacg cgtttattta tttgctctct gatggtattg ccttaaagac

60

tcctgaatgc tgagcatgta ccaaaggag ctgttggtgg cagaagcaaa ccctctccac

120

tggatccaag atgggagctg gggaacctga gacagagaa ggaggttcca gacacacac

180

agcacggttc agagctgcct gaaatgccag gcatcaggc

219

<210> 19

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse Complement Regulatory Element Sequence 04

<400> 19

tctgatttaa taaagtttta tttttccaaa tgcacagctg attgaacctg t

51

<210> 20

<211> 64

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse Complement Regulatory Element Sequence 05

<400> 20

tgagggttta ggctgtgggg aacagaggca gctgccttta ttgaggccta ggggcttggt

60

gact

64

<210> 21

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse Complement Regulatory Element Sequence 06

<400> 21

ttagcacaaa cagtctttat tgggc

25

<210>	<u>22</u>	
<211>	<u>43</u>	
<212>	<u>DNA</u>	
<213>	<u>Artificial Sequence</u>	
<220>		
<223>	<u>Reverse Complement Regulatory Element Sequence 07</u>	
<400>	<u>22</u>	
	<u>actactttaaa cagtctttaa tggaagcagt gtatgagggg ccc</u>	<u>43</u>
<210>	<u>23</u>	
<211>	<u>160</u>	
<212>	<u>DNA</u>	
<213>	<u>Artificial Sequence</u>	
<220>		
<223>	<u>Reverse Complement Regulatory Element Sequence 08</u>	
<400>	<u>23</u>	
	<u>tttgaaaggc tctatatttt tattacagga caaaaccatt cccagcttaa gcatgaacaa</u>	<u>60</u>
	<u>gtctcccgat taacaatggt gaactccatc tggcagcata catggtattg atcatgattc</u>	<u>120</u>
	<u>agtacaaact ctcaagtcct gccattgatt gtatgaatcc</u>	<u>160</u>
<210>	<u>24</u>	
<211>	<u>86</u>	
<212>	<u>DNA</u>	
<213>	<u>Artificial Sequence</u>	
<220>		
<223>	<u>Reverse Complement Regulatory Element Sequence 09</u>	
<400>	<u>24</u>	
	<u>atcaatacaa gtctttattg tcccaaaaaga cacaccaa at cgtacttaat tcaggatctt</u>	<u>60</u>
	<u>gtttctggaa ctggttaact taggtc</u>	<u>86</u>
<210>	<u>25</u>	
<211>	<u>38</u>	
<212>	<u>DNA</u>	
<213>	<u>Artificial Sequence</u>	
<220>		
<223>	<u>Reverse Complement Regulatory Element Sequence 10</u>	
<400>	<u>25</u>	
	<u>aacagtctat ggaggctttt tatttacgtt ttacatct</u>	<u>38</u>
<210>	<u>26</u>	
<211>	<u>54</u>	
<212>	<u>DNA</u>	
<213>	<u>Artificial Sequence</u>	
<220>		

<223>	Reverse Complement Regulatory Element Sequence 11	
<400>	26	
	<u>cactaaagct ctgggatggt tattacaaag gtaaccctga tccttgagat gggc</u>	<u>54</u>
<210>	27	
<211>	30	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Reverse Complement Regulatory Element Sequence 12	
<400>	27	
	<u>caggtttatt gaaaatctga cagaacagat</u>	<u>30</u>
<210>	28	
<211>	33	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Reverse Complement Regulatory Element Sequence 13	
<400>	28	
	<u>tcagttcctg tctttttatt tattcataca caa</u>	<u>33</u>
<210>	29	
<211>	86	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Reverse Complement Regulatory Element Sequence 14	
<400>	29	
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	<u>ctccagcatg tctcctagag accatt</u>	<u>86</u>
<210>	30	
<211>	216	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Reverse Complement Regulatory Element Sequence 15	
<400>	30	
	<u>taagtgaatt taaatgcact ttattttcag acaacctaca cgacatgttc tttcttttta</u>	<u>60</u>
	<u>aaaaaaagaa aagaaaaaaa acaatgtctc cactccaaat aaatcatggt caaaataaaa</u>	<u>120</u>
	<u>gctcaagatg acatcagtcc catttgtcct aagccctggt gttgtatgga tggcaagcag</u>	<u>180</u>
	<u>cagtcagcta tggtgacagg tgacagatcc aatttg</u>	<u>216</u>

<210> 31
 <211> 238
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> A first PB transposase recognition site sequence

<400> 31
 ttaaccctag aaagataatc atattgtgac gtacgttaaa gataatcatg cgtaaaattg 60
 acgcatgtgt tttatcggtc tgtatatcga ggtttattta ttaatttgaa tagatattaa 120
 gttttattat atttacactt acatactaata aataaattca acaacaatt tatttatggtt 180
 tatttattta ttaaaaaaaaa acaaaaactc aaaatttctt ctataaagta acaaaact 238

<210> 32
 <211> 311
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> A second PB transposase recognition site sequence

<400> 32
 tatctataac aagaaaatat atatataata agttatcacg taagtagaac atgaaataac 60
 aatataatta tcgtatgagt taaatcttaa agtcacgta aaagataatc atgcgtcatt 120
 ttgactcacg cggtcgttat agttcaaaat cagtgcactt taccgcattg acaagcacgc 180
 ctacgggag ctccaagcgg cgactgagat gtcctaaatg cacagcgacg gattcgcgct 240
 atttagaaag agagagcaat atttcaagaa tgcatgcgtc aattttacgc agactatctt 300
 tctagggtta a 311

<210> 33
 <211> 594
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> The second nucleic acid encoding a piggyBac transposase

<400> 33
 Met Gly Ser Ser Leu Asp Asp Glu His Ile Leu Ser Ala Leu Leu Gln
 1 5 10 15
 Ser Asp Asp Glu Leu Val Gly Glu Asp Ser Asp Ser Glu Ile Ser Asp
 20 25 30
 His Val Ser Glu Asp Asp Val Gln Ser Asp Thr Glu Glu Ala Phe Ile
 35 40 45
 Asp Glu Val His Glu Val Gln Pro Thr Ser Ser Gly Ser Glu Ile Leu
 50 55 60
 Asp Glu Gln Asn Val Ile Glu Gln Pro Gly Ser Ser Leu Ala Ser Asn
 65 70 75 80
 Arg Ile Leu Thr Leu Pro Gln Arg Thr Ile Arg Gly Lys Asn Lys His
 85 90 95
 Cys Trp Ser Thr Ser Lys Ser Thr Arg Ser Arg Val Ser Ala Leu
 100 105 110

Asn Ile Val Arg Ser Gln Arg Gly Pro Thr Arg Met Cys Arg Asn Ile
 115 120 125
 Tyr Asp Pro Leu Leu Cys Phe Lys Leu Phe Phe Thr Asp Glu Ile Ile
 130 135 140
 Ser Glu Ile Val Lys Trp Thr Asn Ala Glu Ile Ser Leu Lys Arg Arg
 145 150 155 160
 Glu Ser Met Thr Gly Ala Thr Phe Arg Asp Thr Asn Glu Asp Glu Ile
 165 170 175
 Tyr Ala Phe Phe Gly Ile Leu Val Met Thr Ala Val Arg Lys Asp Asn
 180 185 190
 His Met Ser Thr Asp Asp Leu Phe Asp Arg Ser Leu Ser Met Val Tyr
 195 200 205
 Val Ser Val Met Ser Arg Asp Arg Phe Asp Phe Leu Ile Arg Cys Leu
 210 215 220
 Arg Met Asp Asp Lys Ser Ile Arg Pro Thr Leu Arg Glu Asn Asp Val
 225 230 235 240
 Phe Thr Pro Val Arg Lys Ile Trp Asp Leu Phe Ile His Gln Cys Ile
 245 250 255
 Gln Asn Tyr Thr Pro Gly Ala His Leu Thr Ile Asp Glu Gln Leu Leu
 260 265 270
 Gly Phe Arg Gly Arg Cys Pro Phe Arg Met Tyr Ile Pro Asn Lys Pro
 275 280 285
 Ser Lys Tyr Gly Ile Lys Ile Leu Met Met Cys Asp Ser Gly Thr Lys
 290 295 300
 Tyr Met Ile Asn Gly Met Pro Tyr Leu Gly Arg Gly Thr Gln Thr Asn
 305 310 315 320
 Gly Val Pro Leu Gly Glu Tyr Tyr Val Lys Glu Leu Ser Lys Pro Val
 325 330 335
 His Gly Ser Cys Arg Asn Ile Thr Cys Asp Asn Trp Phe Thr Ser Ile
 340 345 350
 Pro Leu Ala Lys Asn Leu Leu Gln Glu Pro Tyr Lys Leu Thr Ile Val
 355 360 365
 Gly Thr Val Arg Ser Asn Lys Arg Glu Ile Pro Glu Val Leu Lys Asn
 370 375 380
 Ser Arg Ser Arg Pro Val Gly Thr Ser Met Phe Cys Phe Asp Gly Pro
 385 390 395 400
 Leu Thr Leu Val Ser Tyr Lys Pro Lys Pro Ala Lys Met Val Tyr Leu
 405 410 415
 Leu Ser Ser Cys Asp Glu Asp Ala Ser Ile Asn Glu Ser Thr Gly Lys
 420 425 430
 Pro Gln Met Val Met Tyr Tyr Asn Gln Thr Lys Gly Gly Val Asp Thr
 435 440 445
 Leu Asp Gln Met Cys Ser Val Met Thr Cys Ser Arg Lys Thr Asn Arg
 450 455 460
 Trp Pro Met Ala Leu Leu Tyr Gly Met Ile Asn Ile Ala Cys Ile Asn
 465 470 475 480
 Ser Phe Ile Ile Tyr Ser His Asn Val Ser Ser Lys Gly Glu Lys Val
 485 490 495
 Gln Ser Arg Lys Lys Phe Met Arg Asn Leu Tyr Met Ser Leu Thr Ser
 500 505 510
 Ser Phe Met Arg Lys Arg Leu Glu Ala Pro Thr Leu Lys Arg Tyr Leu
 515 520 525
 Arg Asp Asn Ile Ser Asn Ile Leu Pro Asn Glu Val Pro Gly Thr Ser

10

530 535 540
Asp Asp Ser Thr Glu Glu Pro Val Met Lys Lys Arg Thr Tyr Cys Thr
545 550 555 560
Tyr Cys Pro Ser Lys Ile Arg Arg Lys Ala Asn Ala Ser Cys Lys Lys
565 570 575
Cys Lys Lys Val Ile Cys Arg Glu His Asn Ile Asp Met Cys Gln Ser
580 585 590
Cys Phe

<210> 34
<211> 943
<212> DNA
<213> Artificial Sequence

<220>
<223> Human EF-1 α gene intron 1

<400> 34
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ccttgaatta cttccacgcc cctggctgca gtacgtgatt cttgatcccg agcttcgggt 120
tggaagtggg tgggagagtt cgaggccttg cgcttaagga gccccttcgc ctctgtcttg 180
agttgaggcc tggcttgggc gctggggccg ccgcgtgcga atctggtggc accttcgcgc 240
ctgtctcgct gctttcgata agtctctagc catttaaaat ttttgatgac ctgctgcgac 300
gctttttttc tggcaagata gtcttgtaaa tgcggggcaa gatctgcaca ctggtatttc 360
ggtttttggg gccgcgggcg gcgacggggc ccgtgcgtcc cagcgcacat gttcggcgag 420
gcggggcctg cgagcgcggc caccgagaat cggacggggg tagtctcaag ctggccggcc 480
tgctctggtg cctggcctcg cgccgccgtg tatcgccccg ccctgggcgg caaggctggc 540
ccggtcggca ccagttgcgt gagcggaaag atggccgctt cccggccctg ctgcagggag 600
ctcaaaatgg aggacgcggc gctcgggaga gcgggcgggt gagtaccca cacaagga 660
aagggccttt ccgtcctcag ccgtcgcttc atgtgactcc acggagtacc gggcgccgtc 720
caggcacctc gattagttct cgagcttttg gagtacgtcg tctttagggtt ggggggaggg 780
gttttatgcg atggagtttc cccacactga gtgggtggag actgaagtta ggccagcttg 840
gcacttgatg taattctcct tgggaatttg cttttttgag tttggatctt ggttcattct 900
caagcctcag acagtgggtc aaagtttttt tcttccattt cag 943

<210> 35
<211> 3619
<212> DNA
<213> Artificial Sequence

<220>
<223> Transcription regulatory element A

<400> 35
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tgcgtgagac tgctgcagag gtcccagctt aggttgtgcc cttctttctt gagaaatgtc 180
ttacttggtg attttgagtg gaaacatgta tttagctgac atatgagcct agtcttttat 240
gtataaatgt gtgttatatt tctagatata aaaatattaa aaattagaaa tcttcagggc 300
tggagagggg ttcattgggt aagagctcat tggttaaggg ctgctcctgt ataggaccg 360
ggttacctgt cagcaccgta tgacggctct caaccatctg cagctcccgt tccagaggac 420
ccagtgtctt cttctggcct ctacagacat acatatagac aaaacaccca tacacaaaa 480

ttttaattaga aatcttaatt tttttctttc aattttctag attgactggg gataactttt 540
 ttgttaactt tactgtcttg aggataacgt tcagtatgag ttgtatttct agagtttgtc 600
 tttattttta ggcaaaaata acctttatta ccattttggg gggtagactgt tttacaactt 660
 ttccaacttt ctgcttcac tcttgtgtcc tatataggcc cctatttact gtcattatta 720
 gagataggac ttgatgtcat gtcaactcca tctttgttat aaatctcaag aagagctaatt 780
 ttcttttgtg ttattacaac caaaaataaa caaggtagct tataaacagt gacttatttt 840
 tatagttcta gatatgggaa gatcatgggt acagtagatt caatgtctag ttggaagttg 900
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 ctgctttat tactgcaaac atacagcttg tggccattgg acttagccat atgagaaatg 1200
 taagaattta ttttatattg tagctgcaaa tggtaggttc atcaaattgt gccttaagtt 1260
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 ttacttagta acagtttgta agtgctactt gatcctgttt tatacttagc attgagtata 1380
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 aataaaaaata cgtaactgaa aaaaatgttt attgcagtgg cctcctctag catggcaaca 2040
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 actaataatc tgtttgaaac tgactgagat agaaagatgt gatgttcctt cccactcact 3360
 ccggattttg atagaagact tgttttattt atttccaaaa ttatatccgc aggaacaag 3420
 ctgtttaaat tcagattatg ctgaagcaaa atggctcctg tatgagaagc aacgtgctgt 3480
 tttacgagca cagagtcctt tttctcataa ctgattgata gtaaatattt tcctgaagaa 3540
 ttattgcaa ccatgaacag tgcaactgtt tcactttttt tccgtgctac ttgctgtacc 3600
 agccattgtc ggttaattaa 3619

<210> 36
 <211> 1114
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Transcription regulatory element B

<400> 36
 gatctgaagt ttggatctgc agaaccaca caaaggccta cgggcttagt agtgtacctg 60
 caatttcagc acttggaagg ctgagaaagg atcccaaggg cagctggcta gctaggctag 120
 tgtagctga gagctctggg ttcgtggagc gactctgggt cagtgaataa gatagagagt 180
 gacatcagct ttgggcttcc acagcaaatg agctcacttg catgcaaaca gaaatgcaaa 240
 cacatgcaca aagcaaaaaca aaaggaacac aggccaaagg tgggtcattc ctataccatc 300
 ccctcagcag ggtgcagtcc ccacaccctg acccagttcc ctcagtatgt tagagaaaat 360
 aactttgccc ctttcaacga acatttcagc tccagagaac ctggcccact ttgaaagctt 420
 taattagaaa tgtgcaatta cccggaacag atgtctgttg tgattgtgga gacataggtt 480
 aaagaatcac acagcagttt gcgtgggttac agaaagggtg caagtaactt taaaacacag 540
 tttttggtaa gtctccaaca tgttacctaa catagcatgg cctcgattac atgtaagcag 600
 tgagtctccg gctgcctggt ttgtgagggt aatgtacttc agcaatagtg ctgaggctgt 660
 acagtgagtg actcatcacc ctaaaaaagt atcgaattcc agtcttcaga gttagctttc 720
 agtaaaacca agtcagtggg gaaatggctc agtaggtaag ggcacccgct gccaaagccca 780
 agacctgtgt cctgtcccgt ggatccagtt ggtggaaaga gagaacggac tcctgcaagg 840
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 ggaagtgggc tcacgcataa ggcactcact ggactctact cttctactct gtggttactt 960
 tttgtgttc aagcatacca taccttgatc tacatgattt ttactccaaa gacacagcca 1020
 gggtaatgtt gtgtgatgga tcagtcttat ttgttacttg ttactagta cttactgaga 1080
 ttgtcgatgg ctttaatgtc aacatgagtg tgga 1114

<210> 37
 <211> 4068
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Transcription regulatory element C

<400> 37
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 tggggctcag ttttctcatc tgtagaaaa ggacacagct gacctgttgg cttctaatag 120
 ttggacagag gctaggattc tgagtctcat tttactacaa atattctttt aatttcttaa 180
 gtcactaaac agcatcagca aggcagggtc gagacatgcg agcaagaatg agattggatt 240
 ctgactcagg tttcaacttg ctgtcaatta ctgacaatgt aagttcattc atcttataga 300
 cttttgtaga acttttgttt ctctccacta taatttcgtt actgttccat attacagtat 360
 gctaaagtta atggtaaaag ttctcacaga attcctagtc ttttctcttt catatttaat 420
 ctcttttctt tcctcctgtc cttactcatt gtgaaattct cttttgtatg catgacttgg 480
 aaacatattt ctttggtggt aaggtagtag gagacaattt attcactttt cacgtatgtc 540
 gtaattggca tattgctgat aaagtttttc aaccatggga acatggtctt gtaagaatta 600
 tttcacattt ttcccagtc aagcccataa tgaaaattga ttctgaattt tttctgtatt 660
 ttttaattct ctgtttgcag ttgtaggaga ataaccctgc agcatctgag agaccaagct 720
 aattacaaga atgactagaa atcctttgca tttttaaaac aattttatac atatgtcact 780
 ttgtctttct aaaaaataaa aataaaaaaa atacctaaga gccgagtttg tgttaaaggc 840

taatgattgt attgtacaat tagtaagaat taaggacaaa ggtctcttta cctgaagttt 900
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 tggtaagacc gtcgatgcac tataaacttg cagaagctga cagcagactg ttcttcactt 1200
 caactcattt atccctttcc tttgggttct gtccaaatca catcaccaga tcacaagaac 1260
 ctaacatcag attgagacgt aaatagatga tatcacattg gatttccacc attgagccac 1320
 accaccagcc acctgcctga taactttcac agtcccagaa gatattatac aagttactag 1380
 ggcaaaaaaga gatcaaagtc tgaatcagct gtgaacccta tgaatggcaa tacctactta 1440
 tcaggcaata caagcccacc cgtgtgatag tggaataaca gtaatatggg caatcactgg 1500
 attgagtcct ggccccactg cagagaatcc atgccaaagca ctgtaaatcc aggaagaaaa 1560
 aaaaaaacct atcactgaag aagacataaa ccctagaaag gaacttacta ctcttactta 1620
 actgagttag caaagcaaca agttatcttc taagtactta tgctggtgct catacacaaa 1680
 attatccatc attcttaatt agagaattct ctctagttaa tggttgtgga ttcaaagact 1740
 cataaatacc aagggtgcta agaattgagc acaattaaga actcagccct aaacaagatt 1800
 tttataacct atcttctaag gctcagaaac attgtggaag aagggtgtcaa aagaatgtaa 1860
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 tgcagttgtt agtgccagga ctgtgtgaca ttgtcactac caacactcag ccttgggtgg 1980
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4068

<210> 38

<211> 3949

<212> DNA

<213> Artificial Sequence

<220>

<223> Transcription regulatory element D

<400> 38

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<u>tgcttataaaa gggaaaggaa acagttaaga atgaggctca gtagttgggg ctttgaac</u>	<u>2460</u>
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<210> 39

<211> 1128

<212> DNA

<213> Artificial Sequence

<220>

<223> Transcription regulatory element E

<400> 39

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<210> 40
 <211> 1352
 <212> DNA
 <213> Artificial Sequence

<220>
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<210> 41
 <211> 1246
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Transcription regulatory element G

<400> 41
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<210> 42

<211> 1886

<212> DNA

<213> Artificial Sequence

<220>

<223> Transcription regulatory element H

<400> 42

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<210> 43
 <211> 1118
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Transcription regulatory element I

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<210> 44
 <211> 1611
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Transcription regulatory element J

<400> 44
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<210> 45

<211> 1299

<212> DNA

<213> Artificial Sequence

<220>

<223> Transcription regulatory element K

<400> 45

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<210> 46

<211> 1108

<212> DNA

<213> Artificial Sequence

<220>

<223> Transcription regulatory element L

<400> 46

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gctgagagct ctgggttcgt ggagcgactc tggttcagt aataagatag agagtgcacat	180
cagctttggg cttccacagc aaatgagctc acttgcatgc aaacagaaat gcaaacacat	240
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atgttgtgtg atggatcagt cttattttgtt acttgtttac tagtacttac tgagattgtc	1080
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<210> 47

<211> 3619

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element A

<400> 47

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gggactctgt gtcgtaaaa cagcacgttg cttctcatc caggaccatt ttgcttcagc	180
ataatctgaa tttaaacagc ttgtttcctg cggatataat ttggaaata aataaaacaa	240
gtcttctatc aaaaatccga gtgagtggga aggaacatca catctttcta tctcagtcag	300
tttcaaacag attattagtc ccaagtaaaa aacagtaata acaaacaca aaaatacaga	360
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aaaaacatga aggttgacaa ttaggtacaa tgtgattata gatttttaaag actaagctga	660
cagtcaataa ccgctgtcca tttaaagcta caataaatat agagctcatc atgttcccca	720
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<210> 48

<211> 1114

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element B

<400> 48

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tgtagatcaa ggtatggtat gcttgaacac caaaaagtaa ccacagagta gaagagtaga 180
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tctggcagaa ttcaggcatg taggtcagag gccaccttgc aggagtcctgt tctctctttc	300
caccaactgg atcccagga caggacacag gtcttgggct tggcagcggg tgcccttacc	360
tactgagcca tttcaccact gacttgggtt tactgaaagc taactctgaa gactggaatt	420
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cattaccctc acaaaccagg cagccggaga ctactgctt acatgtaatc gaggccatgc	540
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tttgtgtggg ttctgcagat ccaaacttca gatc	1114

<210> 49

<211> 4068

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element C

<400> 49

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gctggctcta cttaacttg tgaacagcat cataatgaga tacaggggtg ggggtaagca	180
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gcctgaagtg agtttttaga gccctaaaat tgtttcactt accagcatgg aaatctaaca	480
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<210> 50

<211> 3949

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element D

<400> 50

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<210> 51

<211> 1128

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element E

<400> 51

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<210> 52

<211> 1352

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element F

<400> 52

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taatagtatt tgggtgtggg gttttagaat gtcttttagaa ttacatgaaa tcatagggaa 180
gagaacactt gtgatggaat tggcggcctt aaatgatgaa aaccatagac caagcctgga 240

acacttgctcc tgtccttttg taacattctt gtcattgtgt gtcctctgat ttcttatgat 300
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gaaaacaaat agtccttagag aaataattat agattatcag tggccaagat actgatagaa 480
gcatcaaaga ttattttaaa atgccaggcc agggagatgg ttcagagggt taagaggctt 540
gctgccaagg ctaatgacct gagttctatc ccagaaacca catgcatagt ggaaggatag 600
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<210> 53

<211> 1246

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element G

<400> 53

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<210> 54

<211> 1886

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element H

<400> 54

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tgtccctgtg tctggttcct tcgtagtttg atagtgtttc caaagcttct tcctaaagca      360
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<210> 55

<211> 1118

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element I

<400> 55

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atcatgtaga tcaaggatg gtatgcttga acacaaaaaa gtaaccacag agtagaagag      180
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<210> 56

<211> 1611

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element J

<400> 56

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<210> 57
 <211> 1299
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element K

<400> 57

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<210> 58
 <211> 1108
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Reverse complementary sequence of transcription regulatory element L

<400> 58

ccacactcat gttgacatta aagccatcga caatctcagt aagtactagt aaacaagtaa	60
caaataagac tgatccatca cacaacatta ccctggctgt gtctttggag taaaaatcat	120
gtagatcaag gtatggatat cttgaacacc aaaaagtaac cacagagtag aagagtagag	180
tccagtgagt gccttatgcg tgagccact tccctttttg cgtttgtttt tacttaatgt	240
ctggcagaat tcaggcatgt aggtcagagg ccaccttgca ggagtccgtt ctctctttcc	300
accaactgga tcccaggac aggcacagg tcttgggctt ggcagcgggt gcccttacct	360
actgagccat ttcaccactg acttggtttt actgaaagct aactctgaag actggaattc	420
gatacttttt tagggtgatg agtcactcac tgtacagcct cagcactatt gctgaagtac	480
attaccctca caaaccaggc agccggagac tcaactgctta catgtaatcg aggccatgct	540
atgttaggta acatgttggg gacttaccaa aaactgtgtt ttaaagttac ttgcaacctt	600
ctgtaaccac gcaaatgct gtgtgattct ttaacctatg tctccacaat cacaacagac	660
atctgttccg ggtaattgca catttctaata taaagctttc aaagtgggcc aggttctctt	720

ggagctgaaa tggtcgttga agggggcaaa gttattttct ctaacatcat gaggggaactg 780
ggtcaggggtg tggggactgc accctgctga ggggatggta taggaatgac ccacctttgg 840
cctgtgttcc ttttgttttg ctttgtgcat gtgtttgcat ttctgtttgc atgcaagtga 900
gctcatttgc tgtggaagcc caaagctgat gtcactctct atcttattca ctgaaccaga 960
gtcgtccac gaaccagag ctctcagcta acactagcct agctagccag ctgcccttgg 1020
gatcctttct cagccttcca agtgctgaaa ttgcaggtac actactaagc ccgtaggcct 1080
ttgtgtgggt tctgcagatc caaacttc 1108

<210> 59
 <211> 680
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Human CMV promoter

<400> 59
gttgacattg attattgact agttattaat agtaatcaat tacgggggtca ttagttcata 60
gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgctt ggctgaccgc 120
ccaacgacct cgcgccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag 180
ggactttcca ttgacgtcaa tgggtggagt atttacggt aactgcccac ttggcagtac 240
atcaagtgt tcatatgcc agtacgcccc ctattgacgt caatgacggt aaatggcccg 300
cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg 360
tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat 420
agcgttttga ctacggggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt 480
tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc ccattgacgc 540
aaatgggcgg taggcgtgta cgggtggagg tctatataag cagagctcgt ttagtgaacc 600
gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc 660
gatccagcct ccggactcta 680

<210> 60
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 3'-ITR sequence of a transposon vector

<400> 60
catgctgcaa ttttacgcag actatctttc taggg 35

<210> 61
 <211> 13
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> The sequence of the left terminal repeat of the wildtype piggyBac 5'-ITR

<400> 61
ccctagaaag ata 13

<210> 62

<211> 31
 <212> DNA
 <213> Artificial Sequence

<220>

<223> The sequence of the 31-bp spacer of the wildtype piggyBac 5'-ITR

<400> 62
 atcatattgt gacgtacgtt aaagataatc a 31

<210> 63
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>

<223> The sequence of the left internal repeat of the wildtype piggyBac 5'-ITR

<400> 63
 tgcgtaaaaat tgacgcatg 19

<210> 64
 <211> 13
 <212> DNA
 <213> Artificial Sequence

<220>

<223> The sequence of the right terminal repeat of the wildtype piggyBac 3'-ITR

<400> 64
 tatcttttcta ggg 13

<210> 65
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>

<223> The sequence of the right internal repeat of the wildtype piggyBac 3'-ITR

<400> 65
 catgcgtcaa ttttacgca 19

<210> 66
 <211> 171
 <212> DNA
 <213> Artificial Sequence

<220>

<223> The 5'-internal domain sequence of the PB transposon recognition site

<400> 66
 tgttttatcgt gtctgtatat cgaggtttat ttattaattt gaatagatat taagttttat 60

tatatttaca cttacatact aataataaat tcaacaaaca atttatttat gtttatttat 120
ttattaaaaa aaaacaaaaa ctcaaaattt cttctataaa gtaacaaaac t 171

<210> 67
 <211> 272
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> The 3'-internal domain sequence of the PB transposon recognition site

<400> 67
tatctataac aagaaaatat atatataata agttatcacg taagtagaac atgaaataac 60
aatataatta tcgtatgagt taaatcttaa aagtcacgta aaagataatc atgcgtcatt 120
ttgactcacg cggtcgttat agttcaaaat cagtgcact taccgcattg acaagcacgc 180
ctcacgggag ctccaagcgg cgactgagat gtcctaaatg cacagcgacg gattcgcgct 240
atttagaaaag agagagcaat atttcaagaa tg 272

<210> 68
 <211> 63
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5' inverted terminal repeat sequence

<400> 68
ccctagaaaag ataatcatat tgtgacgtac gttaaagata atcatgcgta aaattgacgc 60
atg 63

<210> 69
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> The sequence of the forward primer of the PCR reaction

<400> 69
gcaaaaaagg gaataagggc gacacgg 27

<210> 70
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> The sequence of the reverse primer of the PCR reaction

<400> 70
catagcccat atatggagtt ccgcgtta 28

<210> 71

<211> 447
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Amino acid sequence of the A chain of PD-L1

<400> 71

Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser
 1 5 10 15
 Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asp Leu
 20 25 30
 Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile Ile Gln
 35 40 45
 Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser Tyr Arg
 50 55 60
 Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn Ala Ala
 65 70 75 80
 Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
 85 90 95
 Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
 100 105 110
 Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
 115 120 125
 Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr Pro Lys
 130 135 140
 Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser Gly Lys
 145 150 155 160
 Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn Val Thr
 165 170 175
 Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr Cys Thr
 180 185 190
 Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
 195 200 205
 Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Thr Pro Arg Asp
 210 215 220
 Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val
 225 230 235 240
 Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr
 245 250 255
 Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu
 260 265 270
 Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln
 275 280 285
 Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser
 290 295 300
 Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys
 305 310 315 320
 Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile
 325 330 335
 Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro
 340 345 350
 Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met

34

355 360 365
Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn
370 375 380
Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr
385 390 395 400
Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn
405 410 415
Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu
420 425 430
His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
435 440 445

<210> 72

<211> 451

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of the heavy chain (HC) of Adalimumab

<400> 72

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val
50 55 60
Glu Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125
Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190
Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205
Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225 230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

35

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350
 Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445
 Pro Gly Lys
450

<210> 73

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of the light chain (LC) of Adalimumab

<400> 73

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr
20 25 30
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
 Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
 Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr
85 90 95
 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala

36
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210