

SEQUENCE LISTING

<110> Hangzhou Geneway Biotechnology Co., Ltd.

<120> Nucleic acid construct for increasing adeno-associated virus yield, and construction method therefor

<130> C21W0497

<150> CN202010826899.8

<151> 2020-08-17

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<160> 22

<170> PatentIn version 3.5

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<211> 14057

<212> DNA

<213> Artificial sequence

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<223> Nucleotide sequence of nucleic acid construct

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 <211> 21
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 <213> Artificial sequence

<220>
 <223> ITR forward primer

<400> 2	
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<210> 3
 <211> 16
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 <213> Artificial sequence

<220>
 <223> ITR reverse primer

<400> 3	
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<210> 4
 <211> 2208
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Cap gene sequence

<400> 4

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gacagcaggg gccttgtgct tcccgggtac aagtacctcg gacccttcaa cggactggac	180
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<210> 5
 <211> 1866
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Rep gene sequence

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caataa	1866

<210> 6

<211> 1388

<212> DNA

<213> Artificial sequence

<220>

<223> Nucleotide sequence of hr1-p6.9-p10 promoter

<400> 6

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tcttgcataa taaaaattta aatgacatca tatttgagaa taacaaatga cattatccct	180
cgattgtgtt ttacaagtag aattctaccc gtaaagcgag tttagttttg aaaaacaaat	240
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tctactcgta aagcgagttc ggttatgagc cgtgtgcaaa acatgacatc agcttatgag	600
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ctgcagtctg gagatacggg cctttaattc aaccaacac aatatattat agttaataa	1320
gaattattat caaatcattt gtatattaat taaaatacta tactgtaaat tacattttat	1380
ttacaatc	1388

<210> 7
 <211> 2202
 <212> DNA

<213> Artificial sequence

<220>

<223> Nucleotide sequence of pH-Acie01

<400> 7

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tatcatgtct ggatctgac actgcttgag cctaggagat ccgatcatgg agataattaa	180
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<210> 8

<211> 11248

<212> DNA

<213> Artificial sequence

<220>

<223> Nucleotide sequence of pFBd-Cap-ITR-Rep

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Met Tyr Asn Ala Asp Ile Gln Ile Asp Ala Lys Leu Gln Cys Gly Val
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Arg Ser Ala Ala Phe Ala Met Ile Asp Asp Lys His Leu Glu Met Tyr
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Lys His Arg Ile Glu Asn Lys Phe Phe Tyr Tyr Tyr Asp Gln Cys Ala
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Asp Ile Ala Lys Pro Asp Arg Leu Pro Asp Asp Asp Gly Ala Cys Cys
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165

170

175

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Leu Tyr Glu Cys Asn Val Cys Lys Glu Ile Ser Thr Asp Glu Arg Phe
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Pro Thr Pro Asp Gly Ala Asp Thr Val Ile Ser Asp Ser Glu Thr Ala
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Ala Ala Ser Asn Phe Leu Ala Ser Val Asn Ser Leu Thr Asp Asn Asp
115 120 125

Leu Val Glu Cys Leu Leu Lys Thr Thr Asp Asn Leu Glu Glu Ala Val
130 135 140

Ser Ser Ala Tyr Tyr Ser Glu Ser Leu Glu Gln Pro Val Val Glu Gln
145 150 155 160

Pro Ser Pro Ser Ser Ala Tyr His Ala Glu Ser Phe Glu His Ser Ala
165 170 175

Gly Val Asn Gln Pro Ser Ala Thr Gly Thr Lys Arg Lys Leu Asp Glu
180 185 190

Tyr Leu Asp Asn Ser Gln Gly Val Val Gly Gln Phe Asn Lys Ile Lys
195 200 205

Leu Arg Pro Lys Tyr Lys Lys Ser Thr Ile Gln Ser Cys Ala Thr Leu
210 215 220

Glu Gln Thr Ile Asn His Asn Thr Asn Ile Cys Thr Val Ala Ser Thr
225 230 235 240

Gln Glu Ile Thr His Tyr Phe Thr Asn Asp Phe Ala Pro Tyr Leu Met
245 250 255

Arg Phe Asp Asp Asn Asp Tyr Asn Ser Asn Arg Phe Ser Asp His Met
260 265 270

Ser Glu Thr Gly Tyr Tyr Met Phe Val Val Lys Lys Ser Glu Val Lys
275 280 285

Pro Phe Glu Ile Ile Phe Ala Lys Tyr Val Ser Asn Val Val Tyr Glu
290 295 300

Tyr Thr Asn Asn Tyr Tyr Met Val Asp Asn Arg Val Phe Val Val Thr
305 310 315 320

Phe Asp Lys Ile Arg Phe Met Ile Ser Tyr Asn Leu Val Lys Glu Thr
325 330 335

Gly Ile Glu Ile Pro His Ser Gln Asp Val Cys Asn Asp Glu Thr Ala
340 345 350

Ala Gln Asn Cys Lys Lys Cys His Phe Val Asp Val His His Thr Phe
355 360 365

Lys Ala Ala Leu Thr Ser Tyr Phe Asn Leu Asp Met Tyr Tyr Ala Gln
370 375 380

Thr Thr Phe Val Thr Leu Leu Gln Ser Leu Gly Glu Arg Lys Cys Gly
385 390 395 400

Phe Leu Leu Ser Lys Leu Tyr Glu Met Tyr Gln Asp Lys Asn Leu Phe

405	410	415
Thr Leu Pro Ile Met Leu Ser Arg Lys Glu Ser Asn Glu Ile Glu Thr		
420	425	430
Ala Ser Asn Asn Phe Phe Val Ser Pro Tyr Val Ser Gln Ile Leu Lys		
435	440	445
Tyr Ser Glu Ser Val Gln Phe Pro Asp Asn Pro Pro Asn Lys Tyr Val		
450	455	460
Val Asp Asn Leu Asn Leu Ile Val Asn Lys Lys Ser Thr Leu Thr Tyr		
465	470	475
Lys Tyr Ser Ser Val Ala Asn Leu Leu Phe Asn Asn Tyr Lys Tyr His		
485	490	495
Asp Asn Ile Ala Ser Asn Asn Asn Ala Glu Asn Leu Lys Lys Val Lys		
500	505	510
Lys Glu Asp Gly Ser Met His Ile Val Glu Gln Tyr Leu Thr Gln Asn		
515	520	525
Val Asp Asn Val Lys Gly His Asn Phe Ile Val Leu Ser Phe Lys Asn		
530	535	540
Glu Glu Arg Leu Thr Ile Ala Lys Lys Asn Lys Glu Phe Tyr Trp Ile		
545	550	555
Ser Gly Glu Ile Lys Asp Val Asp Val Ser Gln Val Ile Gln Lys Tyr		
565	570	575
Asn Arg Phe Lys His His Met Phe Val Ile Gly Lys Val Asn Arg Arg		
580	585	590
Glu Ser Thr Thr Leu His Asn Asn Leu Leu Lys Leu Leu Ala Leu Ile		
595	600	605
Leu Gln Gly Leu Val Pro Leu Ser Asp Ala Ile Thr Phe Ala Glu Gln		
610	615	620

Lys Leu Asn Cys Lys Tyr Lys Lys Phe Glu Phe Asn
625 630 635

<210> 14
<211> 1227
<212> DNA
<213> Unknown

<220>
<223> Nucleotide sequence of Acie02

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agccagccgt cttcaagcag tcgcagccat cgccgtcagg agcggcgtca ggagcagcgt 180
gtcagcgaag aaaacgtgca gattatcggg aacgtcaacg agccgttgac gcgcacctac 240
catcgtcagg gtgtcacgta ttacgtgcac ggtcaggtta acattagcaa tgacgatccg 300
ctattaagtc aagaggatga cgtcatacta attaatagtg aaaatgtgga tcgtgaacgg 360
tttcccgcaca tcaactgccc gcaagtaccag gataacattg cgtcggagac agctgcgcag 420
agggtctctgc aacgagggtt agatcttgag gctcagctga tgaatgagat tgccccaagg 480
tctcccactt atagtccatc ttattcgccg aattacgtaa taccacagtc gccagatttg 540
tttgcctcgc cgcagtctcc gcagccgcag cagcagcagc agcagcaatc agaacccgaa 600
gaagaagtag aggtttcgtg taatatttgt ttactactt ttaaagacac taaaaacgta 660
aattcctcgt ttgtgacttc gattcattgt aaccatgctg tgtgtttcaa gtgttatgtc 720
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cgcggtgtaca ataagcacgg gtatgtagaa tttatgccc ttaacgtcac tcgtaaccag 840
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acggacttga actatgtgga gcaattgcaa aaagaactgt ccgagctgcg agccaagacc 960
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aaatcagaag agttgcaatc gactgtgaat aatctgcaag aacaattgcg taagcaggtg 1140

gccgagtctc aagccaaatt ttcagagttt gagcgcagta actctgattt agtttctaag 1200
 ttacaaactg ttatgtctag acgttaa 1227

<210> 15
 <211> 408
 <212> PRT
 <213> Unknown

<220>
 <223> Amino acid sequence of IE2 protein

<400> 15

Met Ser Arg Gln Ile Asn Ala Ala Thr Pro Ser Ser Ser Arg Arg His
 1 5 10 15

Arg Leu Ser Leu Ser Arg Arg Arg Ile Asn Phe Thr Thr Ser Pro Glu
 20 25 30

Ala Gln Pro Ser Ser Ser Ser Arg Ser Gln Pro Ser Ser Ser Ser Arg
 35 40 45

Ser His Arg Arg Gln Glu Arg Arg Gln Glu Gln Arg Val Ser Glu Glu
 50 55 60

Asn Val Gln Ile Ile Gly Asn Val Asn Glu Pro Leu Thr Arg Thr Tyr
 65 70 75 80

His Arg Gln Gly Val Thr Tyr Tyr Val His Gly Gln Val Asn Ile Ser
 85 90 95

Asn Asp Asp Pro Leu Leu Ser Gln Glu Asp Asp Val Ile Leu Ile Asn
 100 105 110

Ser Glu Asn Val Asp Arg Glu Arg Phe Pro Asp Ile Thr Ala Gln Gln
 115 120 125

Tyr Gln Asp Asn Ile Ala Ser Glu Thr Ala Ala Gln Arg Ala Leu Gln
 130 135 140

Arg Gly Leu Asp Leu Glu Ala Gln Leu Met Asn Glu Ile Ala Pro Arg
 145 150 155 160

Ser Pro Thr Tyr Ser Pro Ser Tyr Ser Pro Asn Tyr Val Ile Pro Gln
165 170 175

Ser Pro Asp Leu Phe Ala Ser Pro Gln Ser Pro Gln Pro Gln Gln Gln
180 185 190

Gln Gln Gln Gln Ser Glu Pro Glu Glu Glu Val Glu Val Ser Cys Asn
195 200 205

Ile Cys Phe Thr Thr Phe Lys Asp Thr Lys Asn Val Asn Ser Ser Phe
210 215 220

Val Thr Ser Ile His Cys Asn His Ala Val Cys Phe Lys Cys Tyr Val
225 230 235 240

Lys Ile Ile Met Asp Asn Ser Val Tyr Lys Cys Phe Cys Ser Ala Thr
245 250 255

Ser Ser Asp Cys Arg Val Tyr Asn Lys His Gly Tyr Val Glu Phe Met
260 265 270

Pro Ile Asn Val Thr Arg Asn Gln Asp Ser Ile Lys Gln His Trp Arg
275 280 285

Glu Leu Leu Glu Asn Asn Thr Val Asn Asn His Thr Thr Asp Leu Asn
290 295 300

Tyr Val Glu Gln Leu Gln Lys Glu Leu Ser Glu Leu Arg Ala Lys Thr
305 310 315 320

Ser Gln Val Glu His Lys Met Thr Met Leu Asn Ser Asp Tyr Ile Met
325 330 335

Leu Lys His Lys His Ala Val Ala Glu Leu Asp Leu Gln Lys Ala Asn
340 345 350

Tyr Asp Leu Gln Glu Ser Thr Lys Lys Ser Glu Glu Leu Gln Ser Thr
355 360 365

Val Asn Asn Leu Gln Glu Gln Leu Arg Lys Gln Val Ala Glu Ser Gln
 370 375 380

Ala Lys Phe Ser Glu Phe Glu Arg Ser Asn Ser Asp Leu Val Ser Lys
 385 390 395 400

Leu Gln Thr Val Met Ser Arg Arg
 405

<210> 16
 <211> 881
 <212> DNA
 <213> Unknown

<220>
 <223> Nucleotide sequence of hr1 of baculovirus

<400> 16
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 aatgaaacaa ataaacaagt tgtcgataaa acattcataa atgacacagc aacatacaat 120
 tcttgcataa taaaaattta aatgacatca tatttgagaa taacaaatga cattatccct 180
 cgattgtgtt ttacaagtag aattctaccc gtaaagcgag tttagttttg aaaaacaaat 240
 gacatcattt gtataatgac atcatcccct gattgtgttt tacaagtaga attctatccg 300
 taaagcgagt tcagttttga aaacaaatga gtcataccta aacacgttaa taatcttctg 360
 atatcagctt atgactcaag ttatgagccg tgtgcaaac atgagataag tttatgacat 420
 catccactga tcgtgcgtta caagtagaat tctactcgta aagccagttc ggttatgagc 480
 cgtgtgcaaa acatgacatc agcttatgac tcatacttga ttgtgtttta cgcgtagaat 540
 tctactcgta aagcgagttc ggttatgagc cgtgtgcaaa acatgacatc agcttatgag 600
 tcataattaa tcgtgcgtta caagtagaat tctactcgta aagcgagttg aaggatcata 660
 tttagttgcg tttatgagat aagattgaaa gcacgtgtaa aatgtttccc gcgcgttggc 720
 acaactattt acaatgcggc caagttataa aagattctaa tctgatatgt tttaaaacac 780
 ctttgcggcc cgagttgttt gcgtacgtga ctagcgaaga agatgtgtgg accgcagaac 840
 agatagtaaa acaaaaccct agtattggag caataatcga t 881

<210> 17
 <211> 990
 <212> DNA
 <213> Unknown

<220>
 <223> Nucleotide sequence of hr2 of baculovirus

<400> 17
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 atgtaaatga tgtcatcggg tctaactcgc tttagagta gaattctacg tgtaaaacat 120
 aatcaagaga tgatgtcatt tgtttttcaa aactgaactc aagaaatgat gtcatttggt 180
 tttcaaaact gaactggctt tacgagtaga attctacttg taaaacacaa tcgagagatg 240
 atgtcatatt ttgcacacgg ctctaattaa actcgcttta cgagtaaaat tctacttgta 300
 acgcatgatc aagggatgat gtcattggat gagtcatttg tttttcaaaa ctaaactcgc 360
 tttagagta gaattctact tgtaaaacac aatcaaggga tgatgtcatt atacaaatga 420
 tgtcatttggt ttttcaaaac taaactcgct ttacgggtag aattctactt gtaaaacagc 480
 aactcgaggg atgatgtcat cttttactcg atgattataa acgtgtttat gtatgactca 540
 tttgtttttc aaaactaaac tcgctttacg agtagaattc tacttgtaac gcacgatcaa 600
 gggatgatgt catttatttg tgcaaagctc gatgtcatct tttgcacacg attataaaca 660
 caatccaaat aatgactcat ttgttttcaa aactgaactc gctttacgag tagaattcta 720
 cttgtaaaac acaatcaagg gatgatgtca ttttcaaaat gatgtcattt gtttttcaaa 780
 actaaactcg ctttacgagt agaattctac ttgtaaaaca caatcaaggg atgatgtcat 840
 tttaaaaatg atcatttggt tttcaaaact aaactcgctt tacgagtaga attctacgtg 900
 taaaacacaa tcaagggatg atgtcattta ctaaataaaa taattattta aataaaactg 960
 ttttttattg tcaaatacac attgattcac 990

<210> 18
 <211> 684
 <212> DNA
 <213> Unknown

<220>
 <223> Nucleotide sequence of hr3 of baculovirus

<400> 18

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acaaatgaca tcatctacct atcatgatca tgtaataat catgttttaa aatgacatca	120
gcttatgact aataattgat cgtgcgttac aagtagaatt ctactcgtaa agcgagttta	180
gttttgaaaa caaatgagtc atcattaaac atgttaataa tcgtgtataa aggatgacat	240
catccactaa tcgtgcgta caagtagaat tctactcgta aagcgagttc ggttttgaaa	300
aacaaatgac atcatttctt gattgtgttt tacacgtaga attctactcg taaagtatgt	360
tcagtttaaa aaacaaatga catcatttta cagatgacat catttcttga ttatgtttta	420
caagtagaat tctactcgta aagcgagttt agttttaaaa aacaaatgac atcatctctt	480
gattatgttt tacaagtaga attctactcg taaagcgagt ttagttttga aaaacaaatg	540
acatcatctc ttgattatgt tttaacaagta gaattctact cgtaaagcga gtttagtttt	600
gaaaaacaaa tgacatcatc ccttgatcat gcgttacaag tagaattcta ctcgtaaagc	660
gagttgaatt ttgattacaa tatt	684

<210> 19
 <211> 675
 <212> DNA
 <213> Unknown

<220>
 <223> Nucleotide sequence of hr4 left of baculovirus

<400> 19	
atgcatataa ttgtgtacaa aatatgactc attaatcgat cgtgcgttac aagtagaatt	60
ctactggtaa agcaagttcg gttgtgagcc gtgtgcaaaa catgacatca taactaatca	120
tgtttataat catgtgcaaa atatgacatc atccgacgat tgtgtttttac aagtagaatt	180
ctactcgtaa agcgagttta aaaattttgt gacgtcaatg aaacaacgtg taatatTTTT	240
tacaatatTT aagtgaacaa ttatgacttc caataatTTT gtggatgtgg atacgtttgc	300
aagacaattg attacagata aatgtagtgc tctaatacgaa agatgcggat ctgttgccgg	360
caaacatttt agagattagt agagaaaggc cagagacaag tattttgagg tgccaactca	420
aaaaaactat gaatacatta aaaaattatt tttaacgaaca aaatatatgg acgattcgat	480
agattataaaa gattttaaca gacgcacatc attgatagtt tttaaattcg ctttaaacaa	540
gagcaccaac tactttccat cgtactaaag agatcatcga ggtggccatt aaacgtttta	600

acaaaattaa ccccgattta aagagttctc cgcgcaatgc ttcagcatta caaatgaatg	660
tttgaaaaat ctaga	675

<210> 20
 <211> 418
 <212> DNA
 <213> Unknown

<220>
 <223> Nucleotide sequence of hr4 right of baculovirus

<400> 20	
aactggcttt acgagtagaa ttctacttgt aaaacacaat caagaaatga tgtcattttt	60
gtacgtgatt ataaacatgt ttaaacaatgg tacattgaac ttaatTTTTg caagttgata	120
aactagatta atgtatgact ctttgtttg tgcaagttga taaacgtgat taatatatga	180
ctcatatgtt tgtgcaaaaa tgggtgtcatc gtacaaactc gctttacgag tagaattcta	240
cttgtaaaac acaatcgagg gatgatgtca tttgtagaat gatgtcattt gttttttcaa	300
aaccgaactc gctttacgag tagaattcta cttgtaaaac acaatcgagg gatgatgtca	360
tttgtagaat gatgtcatcg tacaaactcg ctttacgagt agaattctag taaaacac	418

<210> 21
 <211> 725
 <212> DNA
 <213> Unknown

<220>
 <223> Nucleotide sequence of hr5 of baculovirus

<400> 21	
ttgaaaattt attgcctaatt attatTTTTg tcagttcgtt gtcattttatt aatttggatg	60
atgtccattt gtttttaaaa ttgaactggc tttacgagta gaattctacg cgtaaaacac	120
aatcaagtat gagtcataag ctgatgtcat gttttgcaca cggctcataa ccgaactggc	180
tttacgagta gaattctact tgtaacgcac gatcgagtgg atgatgggtca tttgtttttc	240
aaatcgagat gatgtcatgt tttgcacacg ggctcataaa ctgcttttac agtagaattc	300
tacgtgtaac gcacgatcga ttgatgagtc atttgttttg caatatgata tcatacaata	360
tgactcattt gtttttcaaa accgaacttg atttacgggt agaattctac tcgtaaagca	420

caatcaaaaa gatgatgtca tttgtttttc aaaactgaac tctcggcttt acgagtagaa	480
ttctacgtgt aaaacacaat caagaaatga tgtcatttgt tataaaaaata aaagctgatg	540
tcatgttttg cacatggctc ataactaaac tcgcttttac ggtagaattc tacgcgtaaa	600
acatgattga taattaaata attcatttgc aaagctatac gttaaataca acggacgtta	660
tggaattgta taatattaaa tatgcaattg atccaacaaa taaaattata atagagcaag	720
tcgac	725

<210> 22
 <211> 4400
 <212> DNA
 <213> Artificial sequence

<220>
 <223> pAAV-EGFP plasmid

<400> 22	
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ttttccatag gctccgcccc cctgacgagc atcacaaaaa tcgacgctca agtcagaggt	120
ggcgaaaccc gacaggacta taaagatacc aggcgtttcc ccctggaagc tccctcgtgc	180
gctctcctgt tccgaccctg ccgcttaccg gatacctgtc cgcctttctc ccttcgggaa	240
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ccaagctggg ctgtgtgcac gaaccccccg ttcagcccga ccgctgcgcc ttatccggta	360
actatcgtct tgagtccaac ccggtgaagac acgacttatt gccactggca gcagccactg	420
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ccttcggaaa aagagttggt agctcttgat ccggcaaaca aaccaccgct ggtagcgggtg	600
gtttttttgt ttgcaagcag cagattacgc gcagaaaaaa aggatctcaa gaagatcctt	660
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tgtagataac tacgatacgg gagggcttac catctggccc cagtgctgca atgataccgc	960

gagacccacg ctcaccggct ccagatttat cagcaataaa ccagccagcc ggaagggccg	1020
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aagctagagt aagtagttcg ccagttaata gtttgcgcaa cgttgttgcc attgctacag	1140
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aagtttaaac aggaaccctt agtgatggag ttggccactc cctctctgcg cgctcgctcg	4320
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agcgcgcagc tgcctgcagg	4400