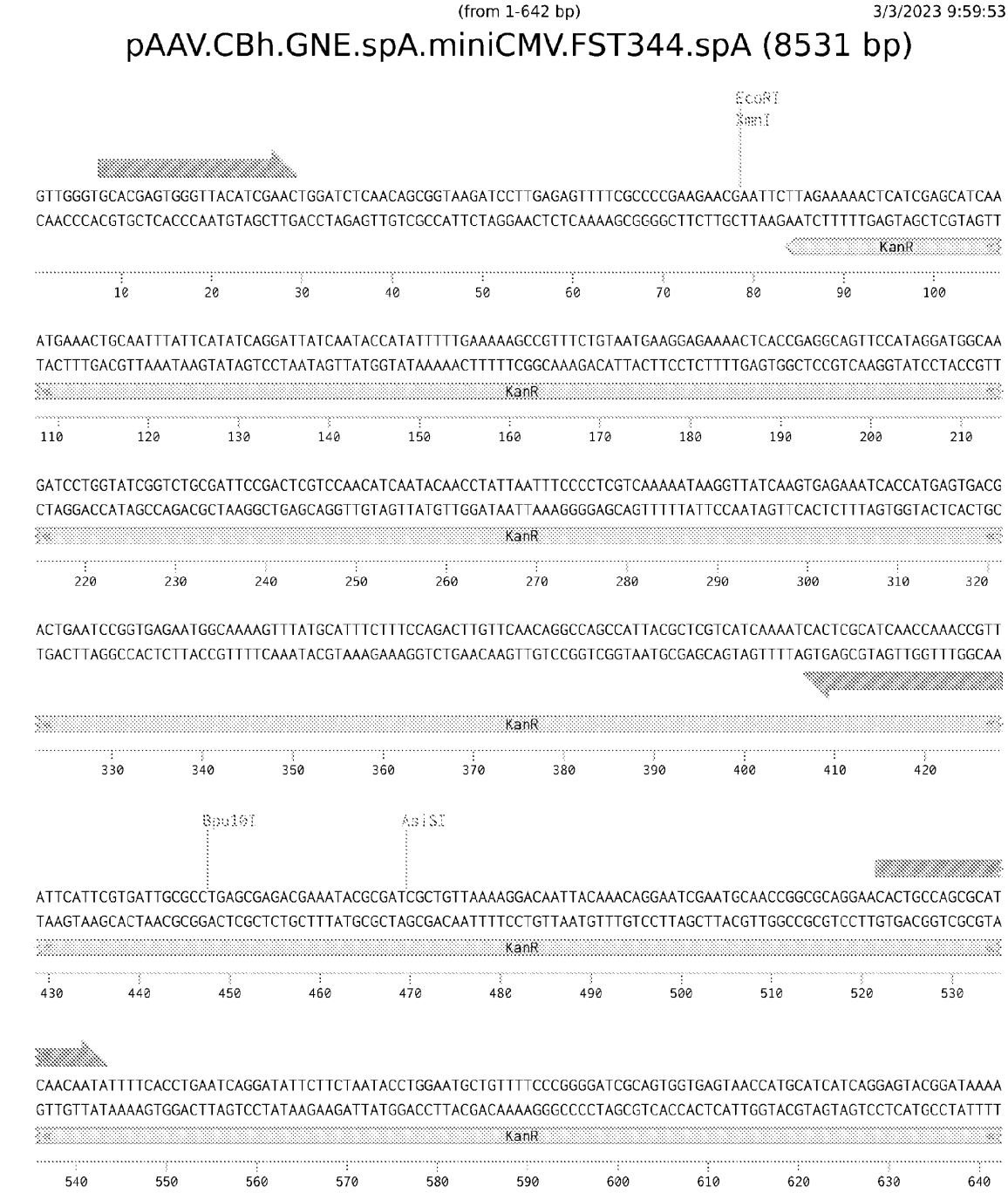


https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

FIG. 1



https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

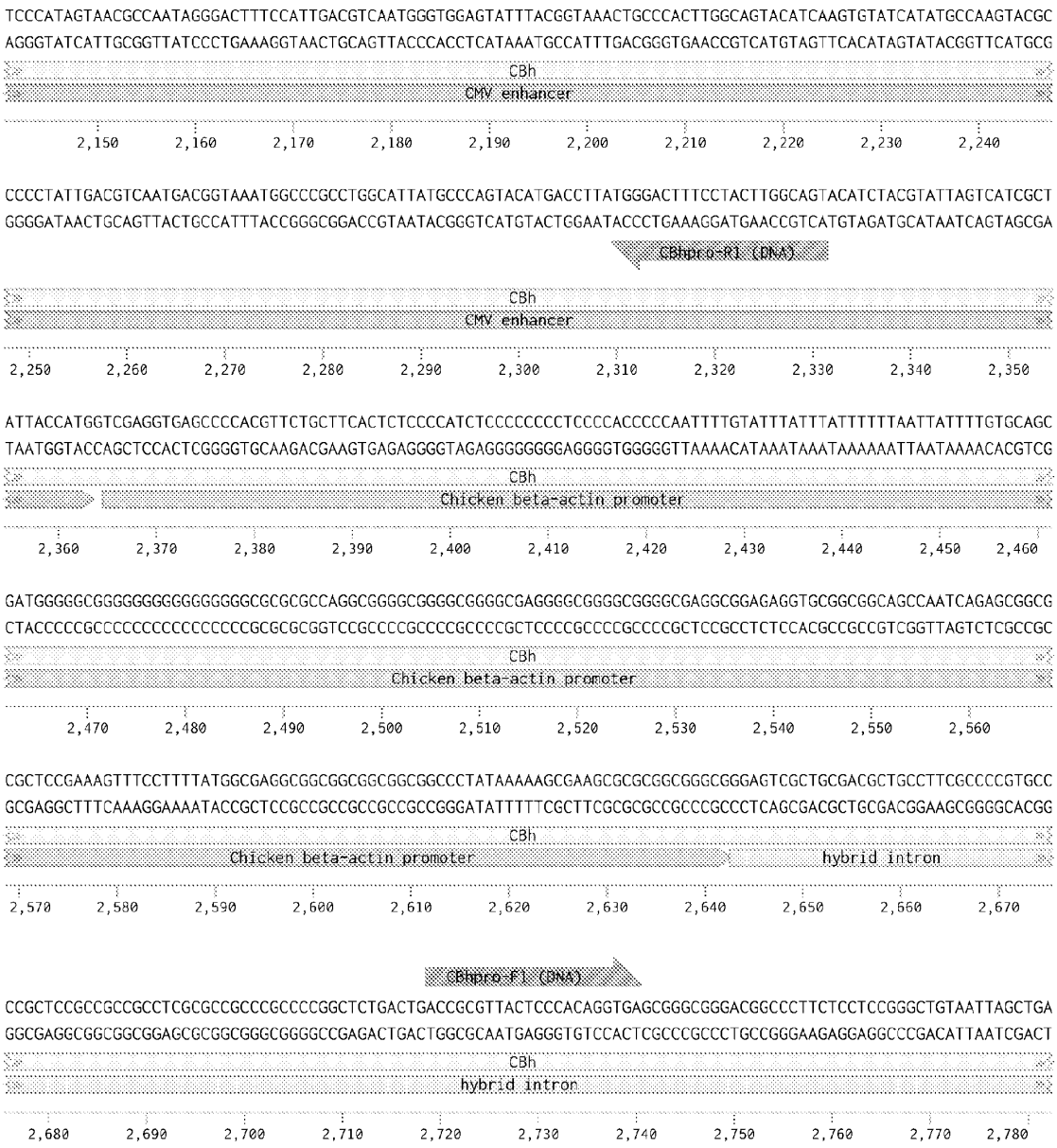
FIG. 1 (cont.)



https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

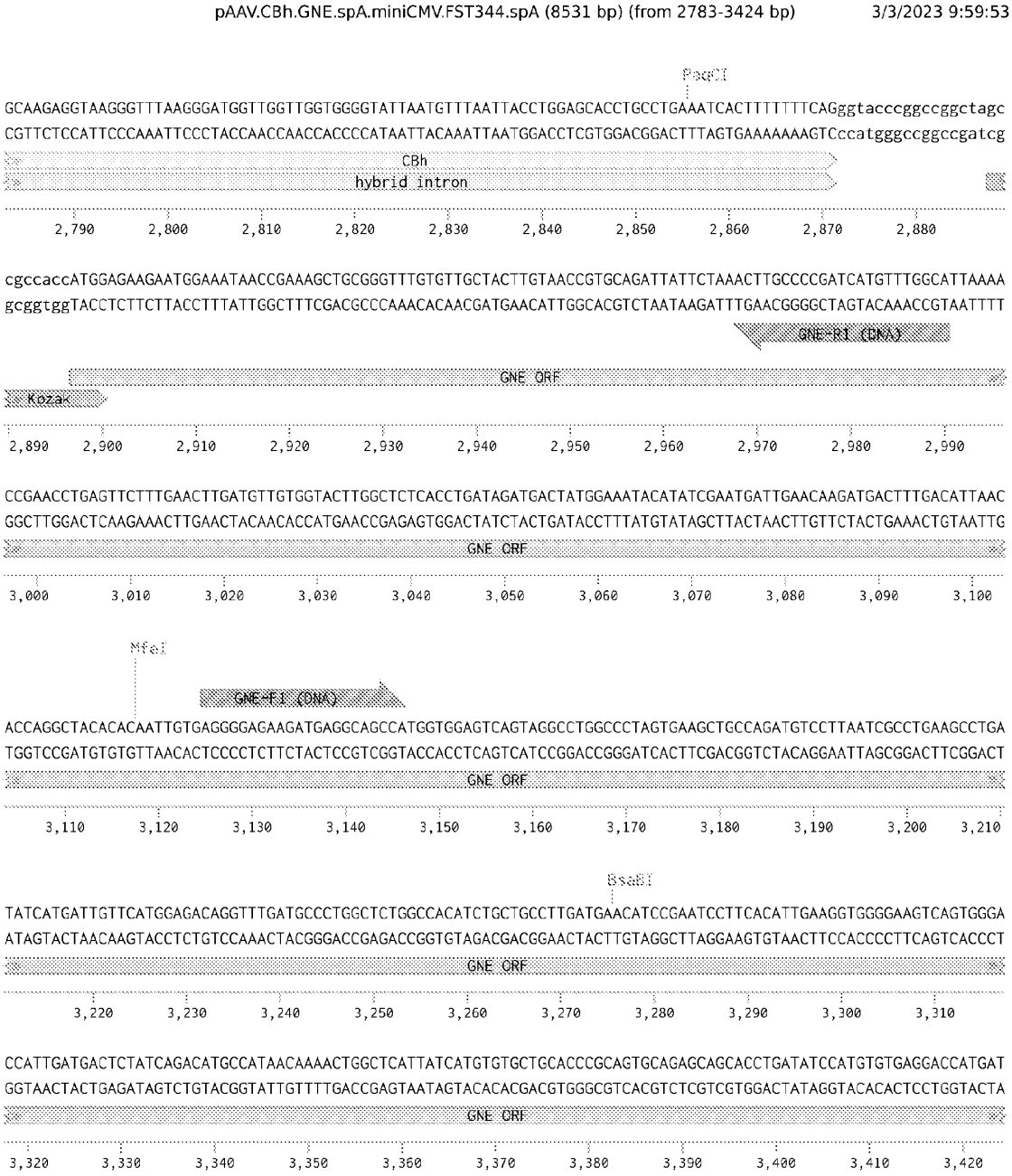
FIG. 1 (cont.)

pAAV.CBh.GNE.spA.miniCMV.FST344.spA (8531 bp) (from 2141-2782 bp) 3/3/2023 9:59:53 AM



https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

FIG. 1 (cont.)



https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

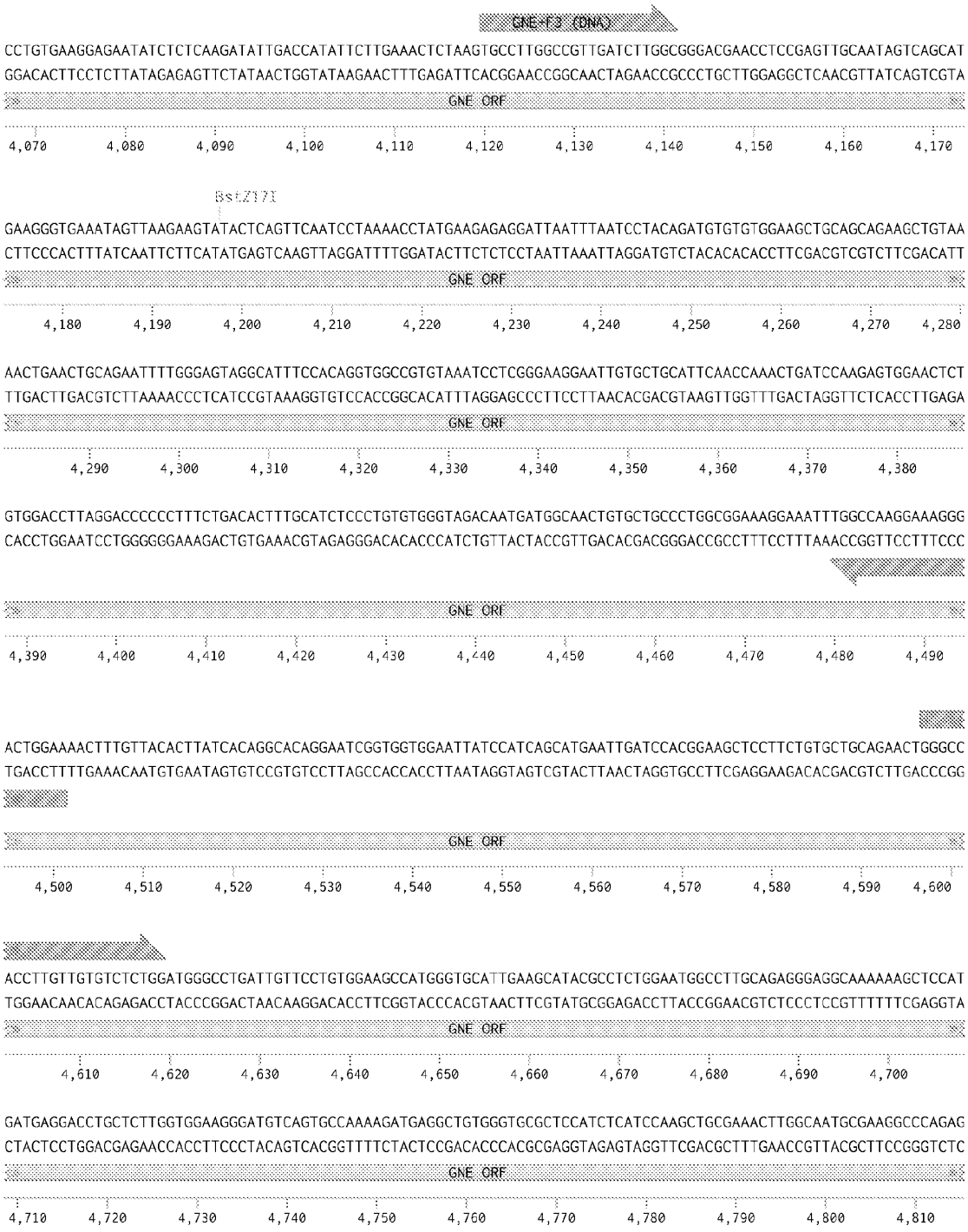
FIG. 1 (cont.)



https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

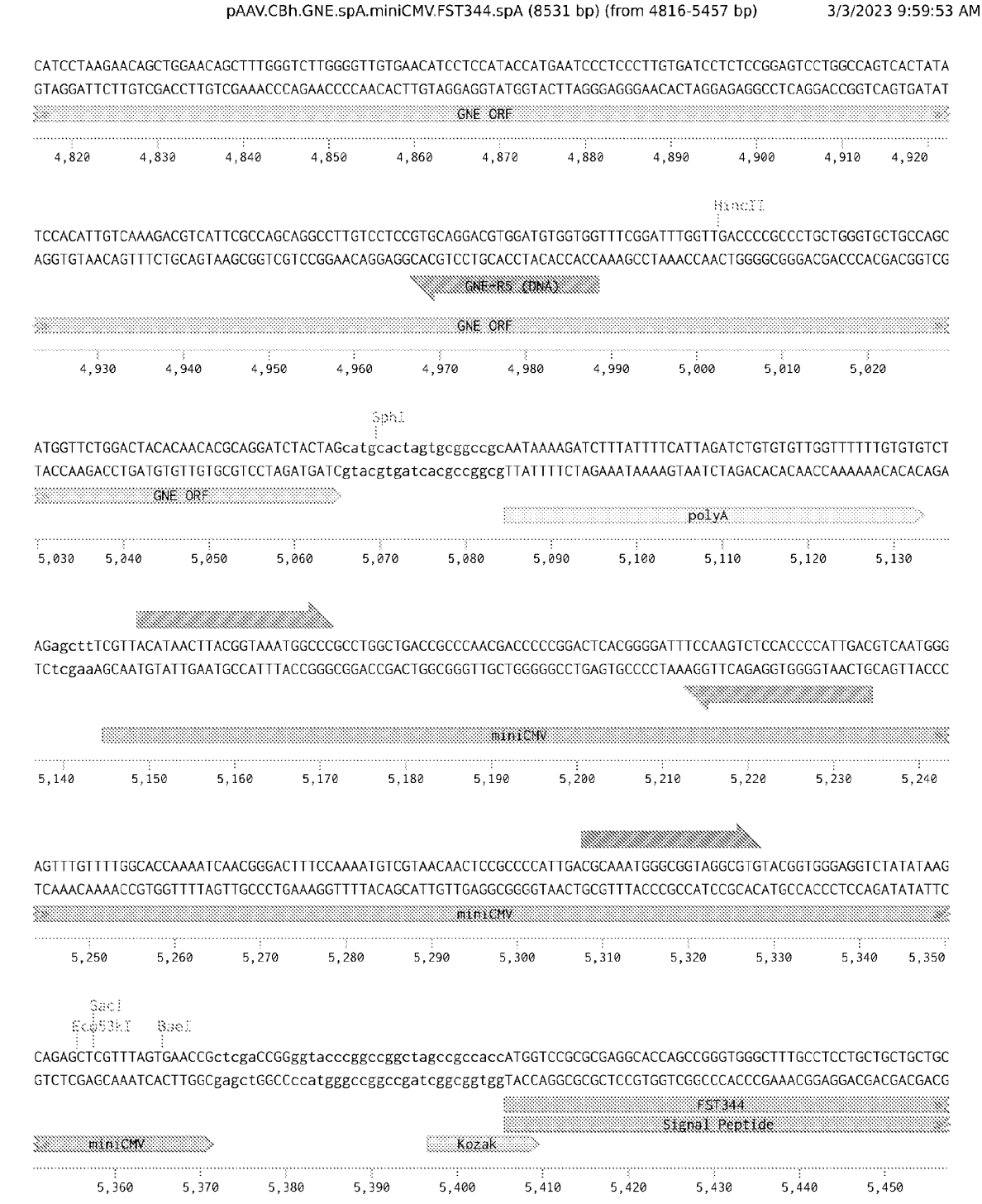
FIG. 1 (cont.)

pAAV.CBh.GNE.spA.miniCMV.FST344.spA (8531 bp) (from 4067-4815 bp) 3/3/2023 9:59:53 AM



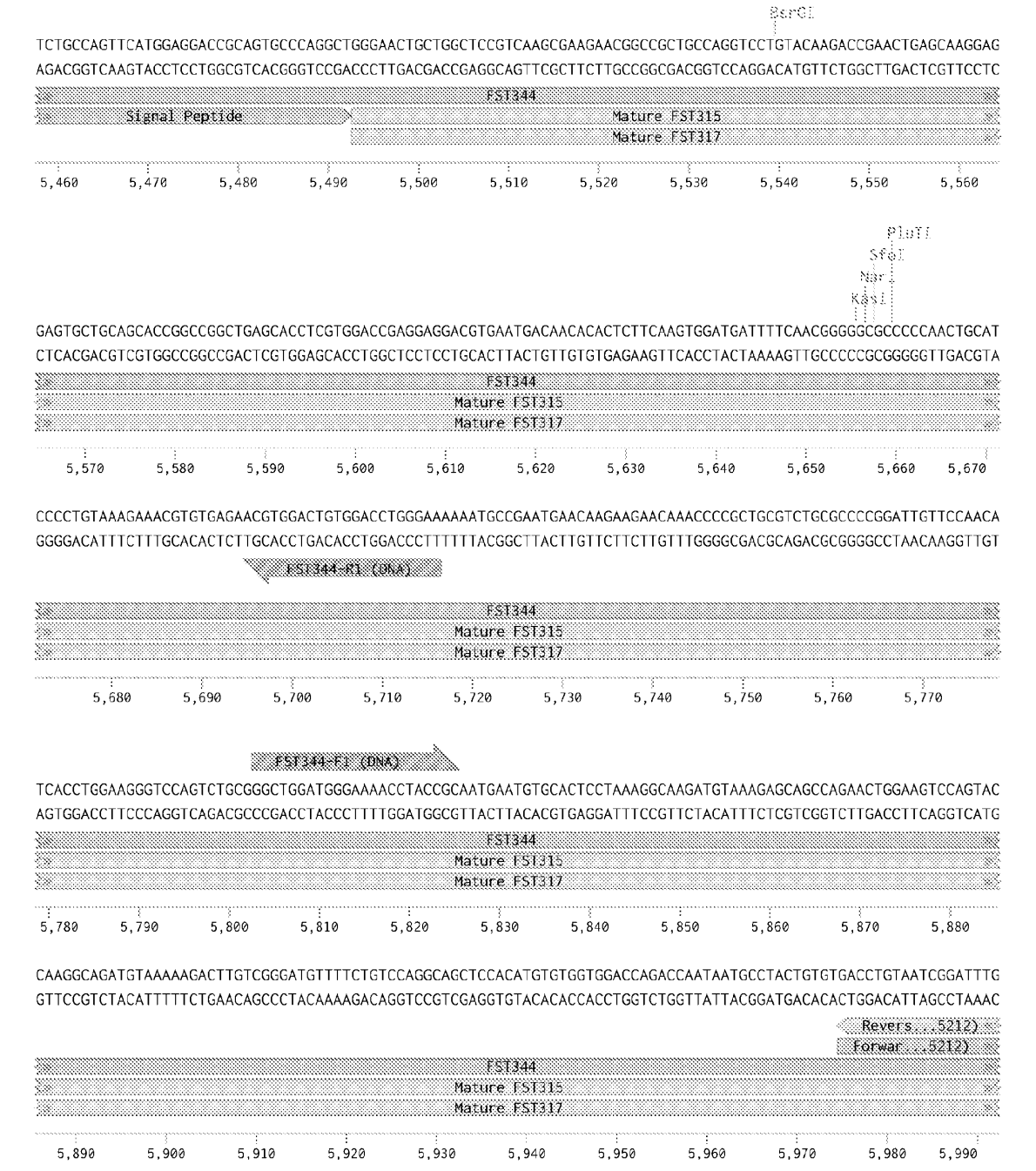
https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

FIG. 1 (cont.)



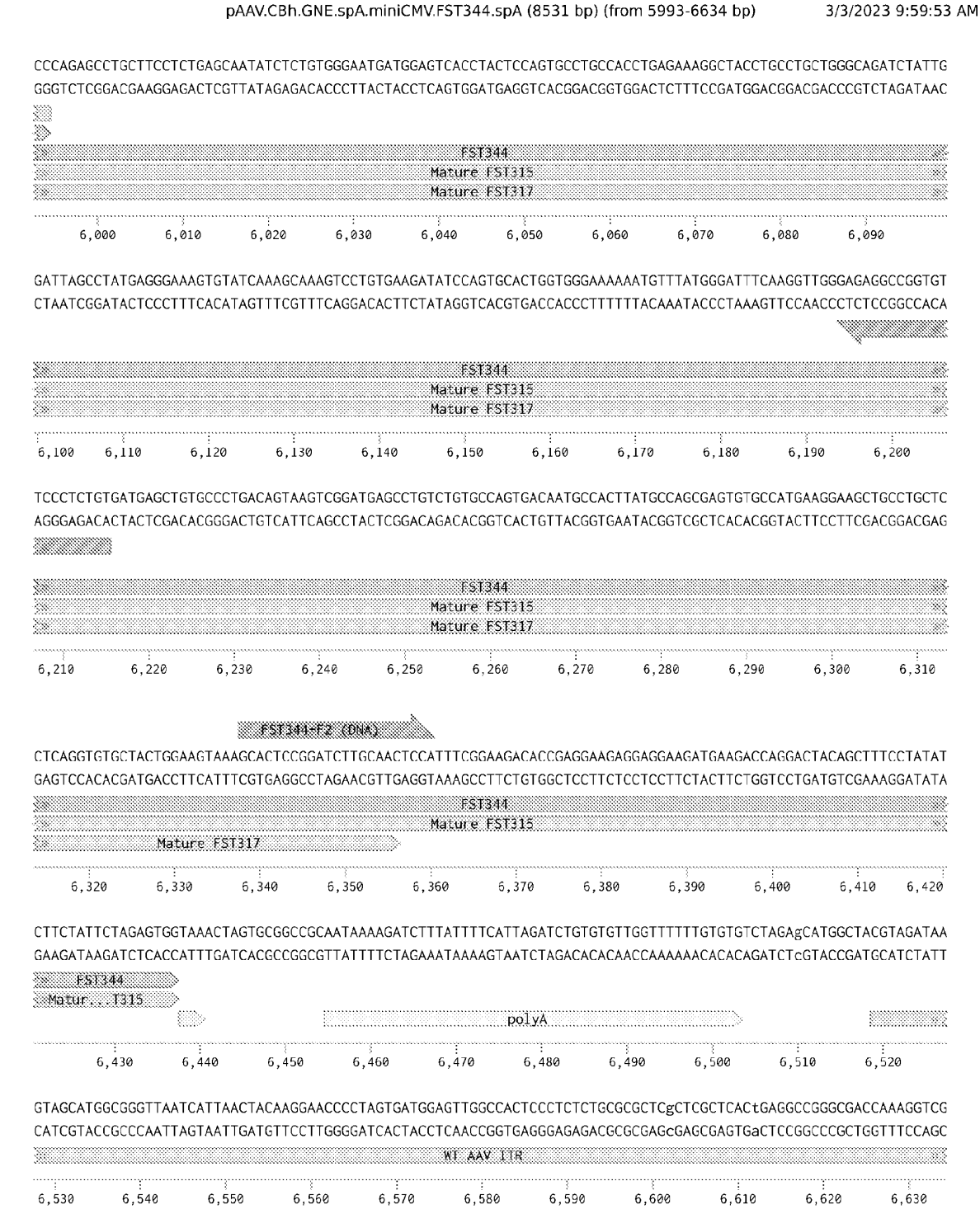
https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

FIG. 1 (cont.)



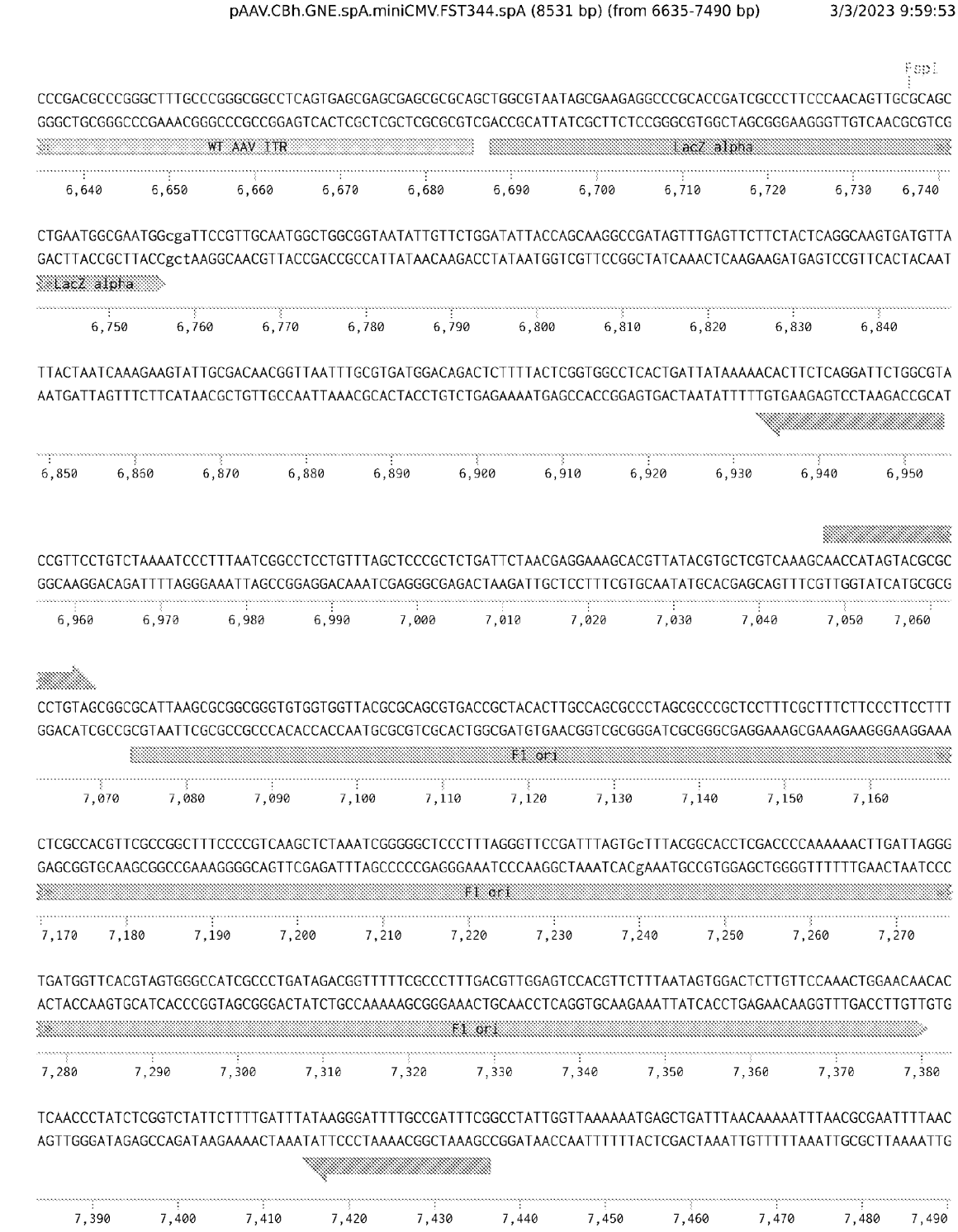
https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

FIG. 1 (cont.)



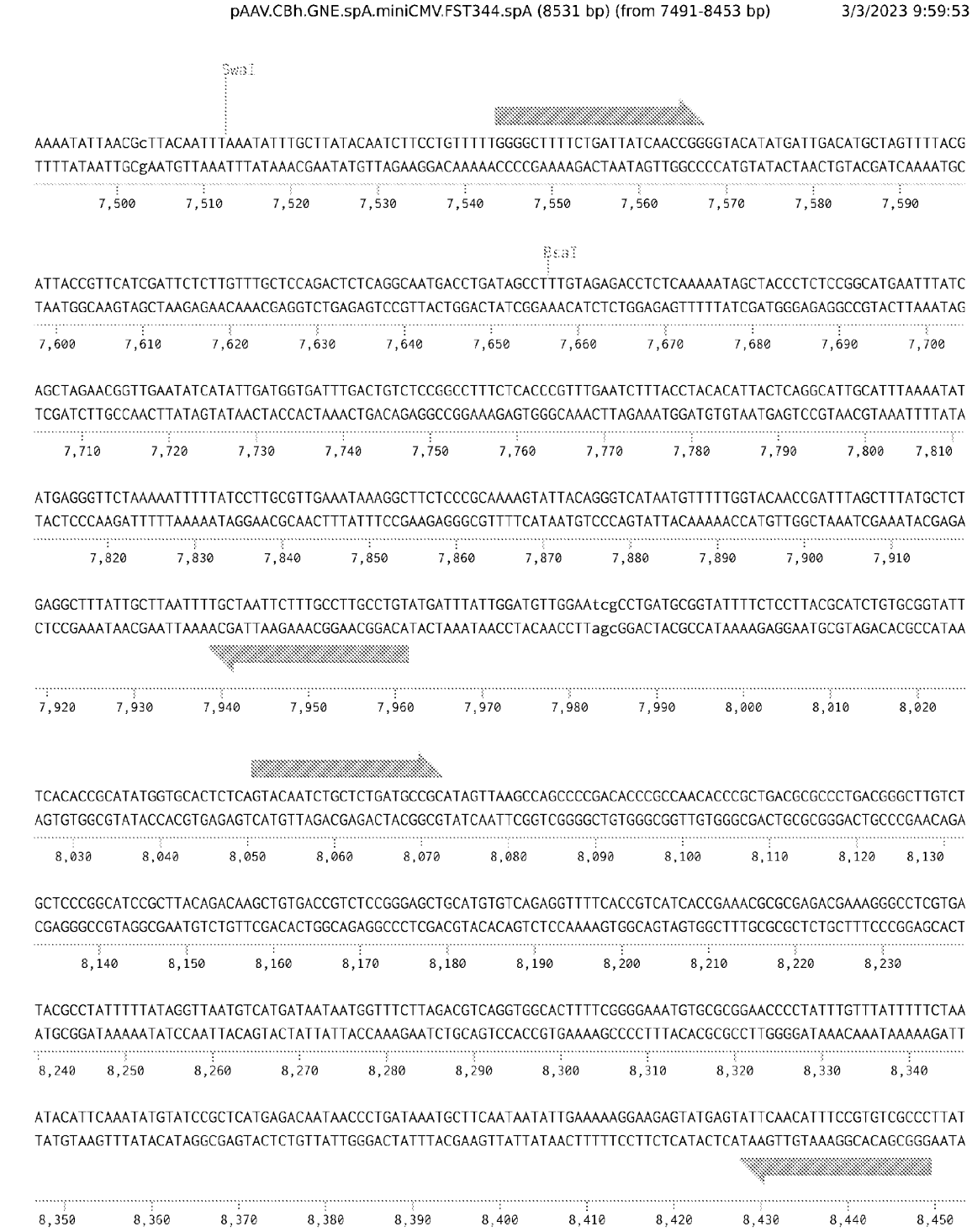
https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

FIG. 1 (cont.)



https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

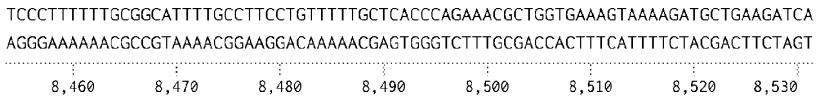
FIG. 1 (cont.)



https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

FIG. 1 (cont.)

pAAV.CBh.GNE.spA.miniCMV.FST344.spA (8531 bp) (from 8454-8531 bp) 3/3/2023 9:59:53 AM



https://benchling.com/patricialam/f/lib_d784nIWW-gne/seq_gaO3c6jq-paav-cbh-gne-spa-minicmv-fst344-spa/edit

FIG. 1 (cont.)

pAAV.CMV.GNE.spA.miniCM

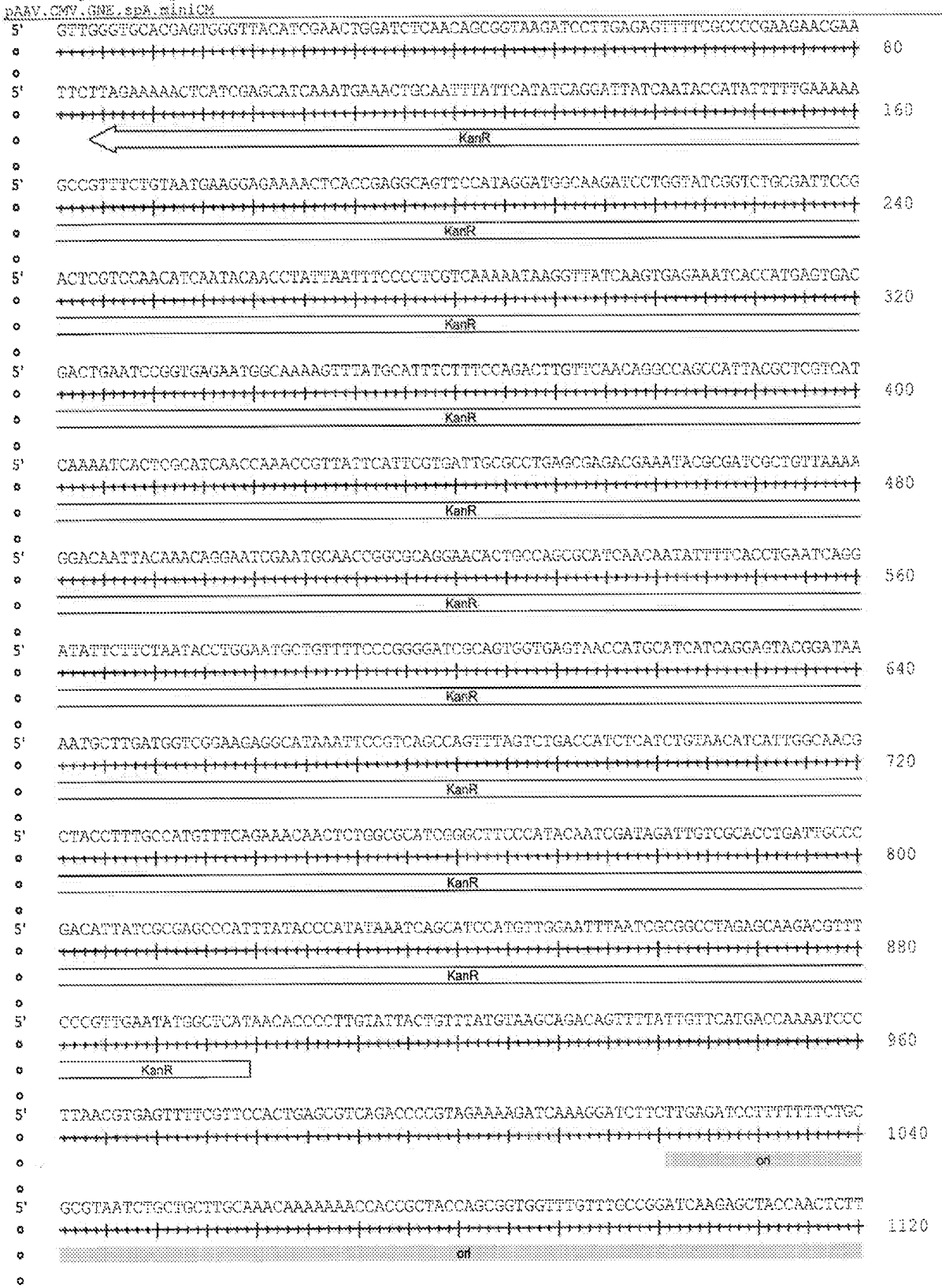


FIG. 2

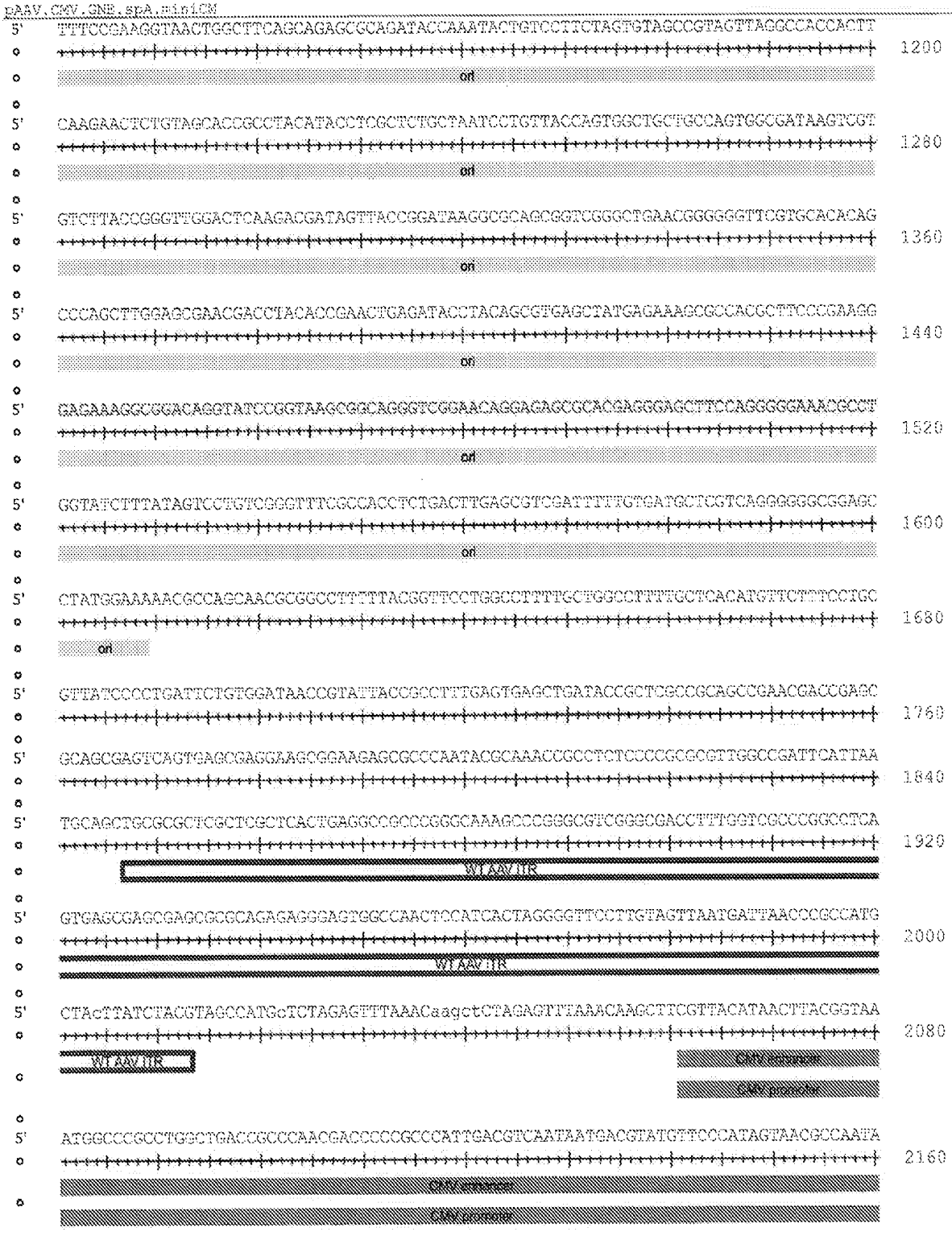

5' TTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT
1200
ori
5' CAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGT
1280
ori
5' GTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAG
1360
ori
5' CCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGG
1440
ori
5' GAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCT
1520
ori
5' GGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGC
1600
ori
5' CTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGC
1680
ori
5' GTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGC
1760
5' GCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAA
1840
5' TGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCA
1920
WT AAV ITR
5' GTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAGTTAATGATTAACCCGCCATG
2000
WT AAV ITR
5' CTACTTATCTACGTAGCCATGcTCTAGAGTTTAAACaagctCTAGAGTTTAAACAAGCTTCGTTACATAACTTACGGTAA
2080
WT AAV ITR
CMV enhancer
CMV promoter
5' ATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATA
2160
CMV enhancer
CMV promoter

FIG. 2 (cont.)

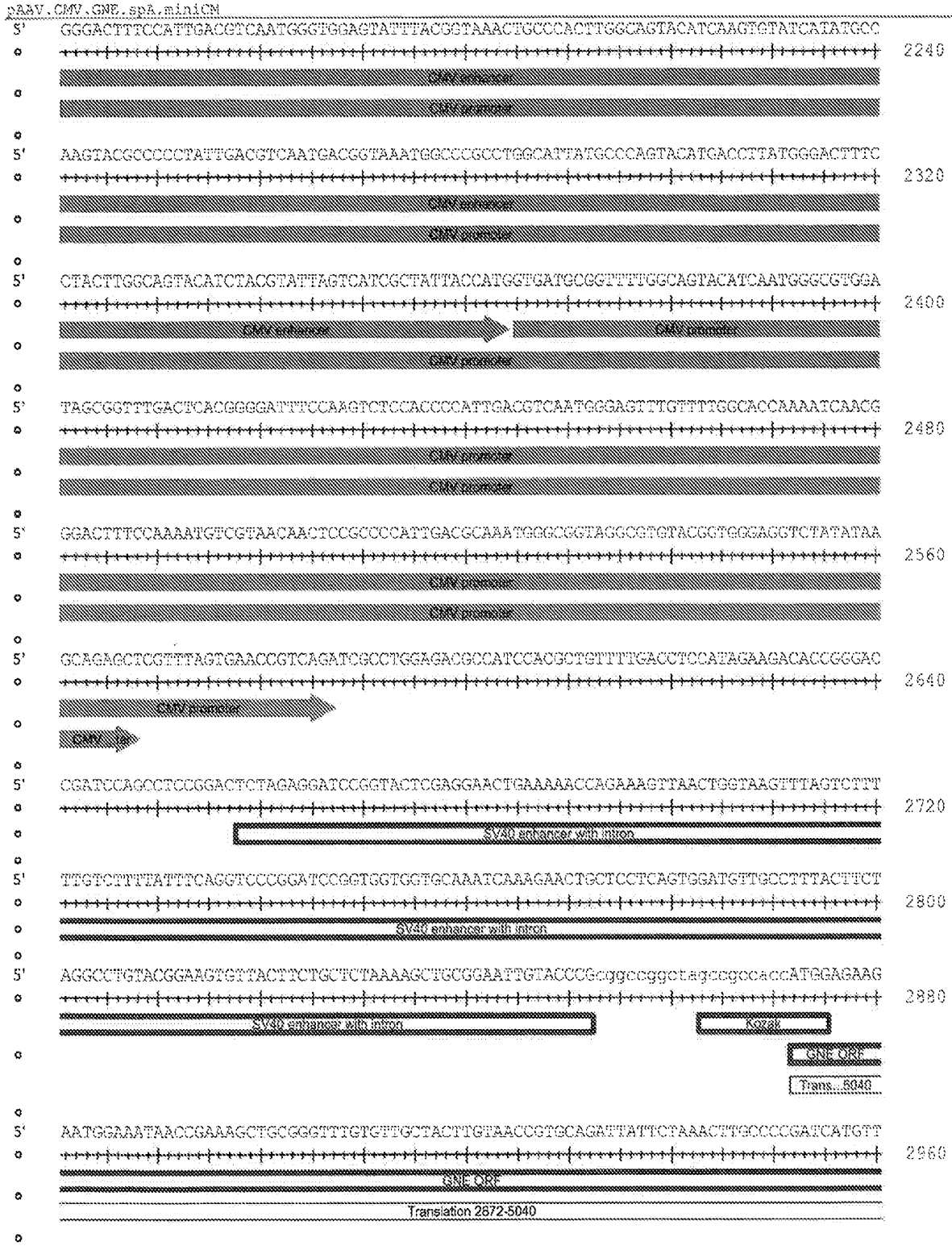

5' GGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCC
2240
CMV enhancer
CMV promoter
5' AAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC
2320
CMV enhancer
CMV promoter
5' CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGA
2400
CMV enhancer
CMV promoter
CMV promoter
5' TAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACG
2480
CMV enhancer
CMV promoter
5' GGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAA
2560
CMV promoter
CMV promoter
5' GCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGAC
2640
CMV promoter
CMV Ie
5' CGATCCAGCCTCCGGACTCTAGAGGATCCGGTACTCGAGGAACTGAAAAACCAGAAAGTTAACTGGTAAGTTTAGTCTTT
2720
SV40 enhancer with intron
5' TTGTCTTTTATTTCAGGTCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTGGATGTTGCCTTTACTTCT
2800
SV40 enhancer with intron
5' AGGCCTGTACGGAAGTGTTACTTCTGCTCTAAAAGCTGCGGAATTGTACCCGcggccggctagccgccaccATGGAGAAG
2880
SV40 enhancer with intron
Kozak
GNE ORF
Trans...6040
5' AATGGAAATAACCGAAAGCTGCGGGTTTGTGTTGCTACTTGTAACCGTGCAGATTATTCTAAACTTTGCCCCGATCATGTT
2960
GNE ORF
Translation 2672-5040

FIG. 2 (cont.)

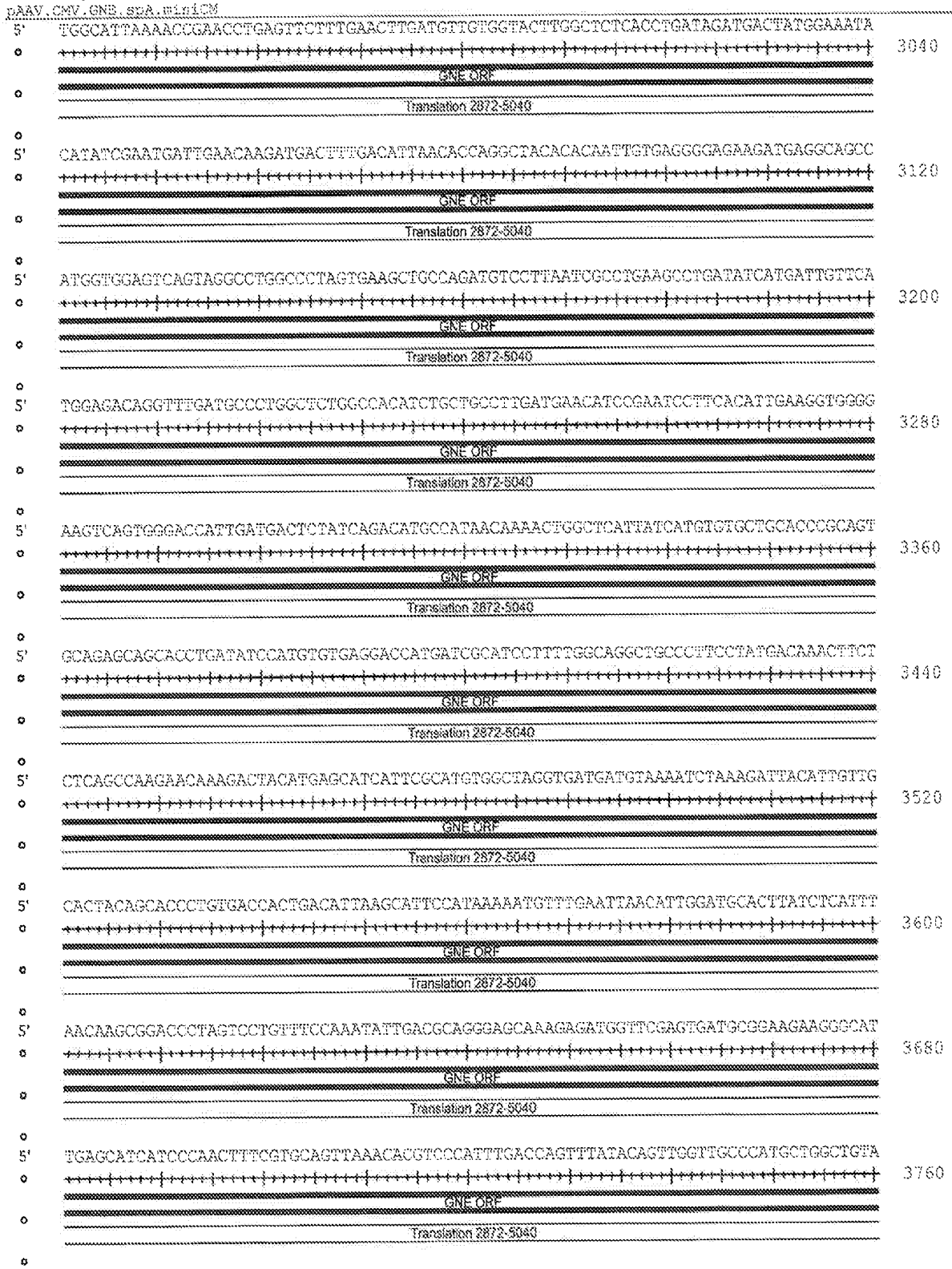

5' TGGCATTAAAACCGAACCTGAGTTCTTTGAACTTGATGTTGTGGTACTTGGCTCTCACCTGATAGATGACTATGGAAATA
3040
GNE ORF
Translation 2872-5040
5' CATATCGAATGATTGAACAAGATGACTTTGACATTAACACCAGGCTACACACAATTGTGAGGGGAGAAGATGAGGCAGCC
3120
GNE ORF
Translation 2872-5040
5' ATGGTGGAGTCAGTAGGCCTGGCCCTAGTGAAGCTGCCAGATGTCCTTAATCGCCTGAAGCCTGATATCATGATTGTTCA
3200
GNE ORF
Translation 2872-5040
5' TGGAGACAGGTTTGATGCCCTGGCTCTGGCCACATCTGCTGCCTTGATGAACATCCGAATCCTTCACATTGAAGGTGGGG
3280
GNE ORF
Translation 2872-5040
5' AAGTCAGTGGGACCATTGATGACTCTATCAGACATGCCATAACAAAACTGGCTCATTATCATGTGTGCTGCACCCGCAGT
3360
GNE ORF
Translation 2872-5040
5' GCAGAGCAGCACCTGATATCCATGTGTGAGGACCATGATCGCATCCTTTTGGCAGGCTGCCCTTCCTATGACAAACTTCT
3440
GNE ORF
Translation 2872-5040
5' CTCAGCCAAGAACAAAGACTACATGAGCATCATTCGCATGTGGCTAGGTGATGATGTAAAATCTAAAGATTACATTGTTG
3520
GNE ORF
Translation 2872-5040
5' CACTACAGCACCCTGTGACCACTGACATTAAGCATTCCATAAAAATGTTTGAATTAACATTGGATGCACTTATCTCATTT
3600
GNE ORF
Translation 2872-5040
5' AACAAGCGGACCCTAGTCCTGTTTCCAAATATTGACGCAGGGAGCAAAGAGATGGTTCGAGTGATGCGGAAGAAGGGCAT
3680
GNE ORF
Translation 2872-5040
5' TGAGCATCATCCCAACTTTCGTGCAGTTAAACACGTCCCATTTGACCAGTTTATACAGTTGGTTGCCCATGCTGGCTGTA
3760
GNE ORF
Translation 2872-5040

FIG. 2 (cont.)

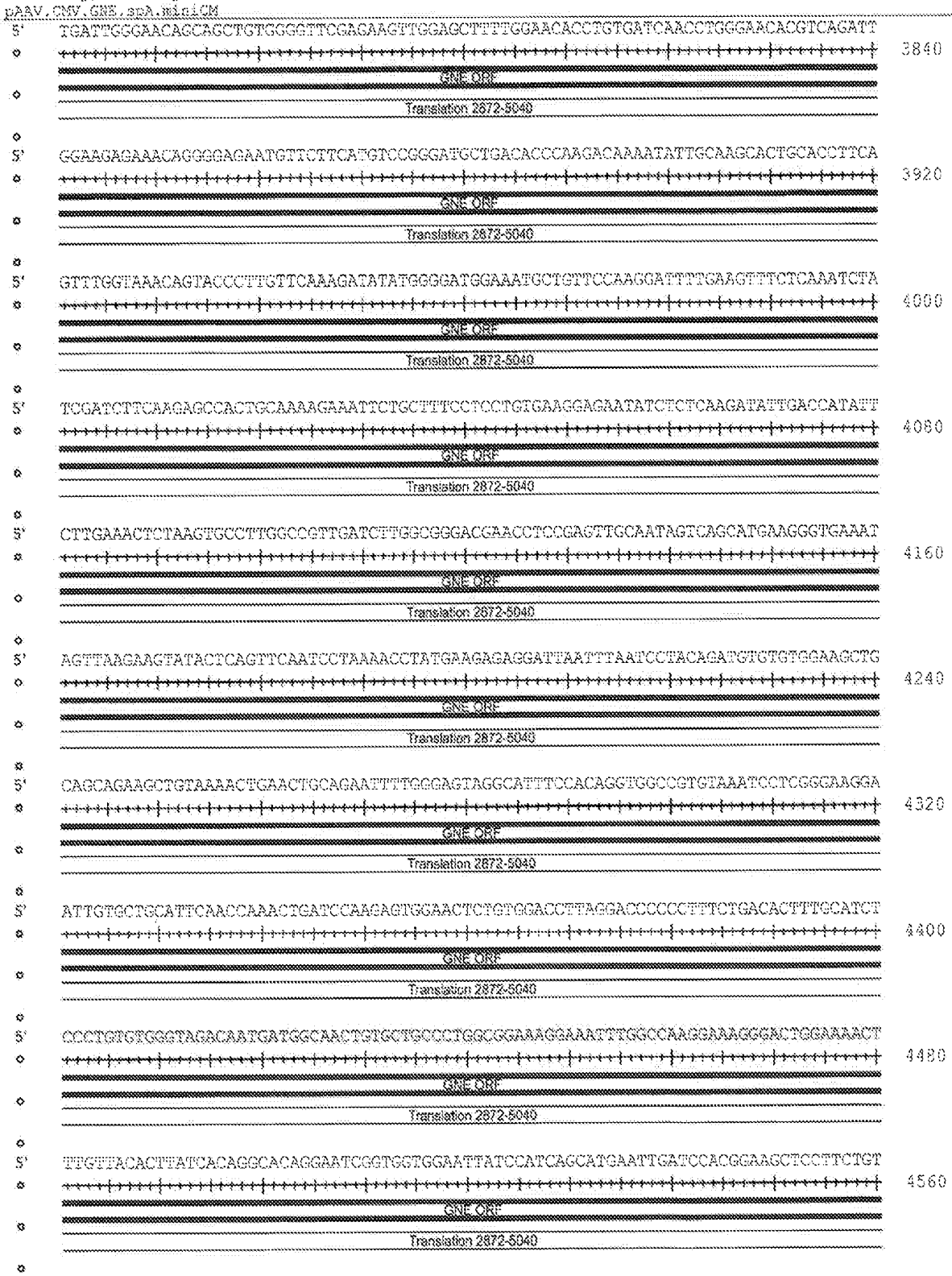

5' TGATTGGGAACAGCAGCTGTGGGGTTCGAGAAGTTGGAGCTTTTGGAACACCTGTGATCAACCTGGGAACACGTCAGATT
3840
GNE ORF
Translation 2872-5040
5' GGAAGAGAAACAGGGGAGAATGTTCTTCATGTCCGGGATGCTGACACCCAAGACAAAATATTGCAAGCACTGCACCTTCA
3920
GNE ORF
Translation 2872-5040
5' GTTTGGTAAACAGTACCCTTGTTCAAAGATATATGGGGATGGAAATGCTGTTCCAAGGATTTTGAAGTTTCTCAAATCTA
4000
GNE ORF
Translation 2872-5040
5' TCGATCTTCAAGAGCCACTGCAAAAGAAATTCTGCTTTCCTCCTGTGAAGGAGAATATCTCTCAAGATATTGACCATATT
4080
GNE ORF
Translation 2872-5040
5' CTTGAAACTCTAAGTGCCTTGGCCGTTGATCTTGGCGGGACGAACCTCCGAGTTGCAATAGTCAGCATGAAGGGTGAAAT
4160
GNE ORF
Translation 2872-5040
5' AGTTAAGAAGTATACTCAGTTCAATCCTAAAACCTATGAAGAGAGGATTAATTTAATCCTACAGATGTGTGTGGAAGCTG
4240
GNE ORF
Translation 2872-5040
5' CAGCAGAAGCTGTAAAACTGAACTGCAGAATTTTGGGAGTAGGCATTTCCACAGGTGGCCGTGTAAATCCTCGGCAAGGA
4320
GNE ORF
Translation 2872-5040
5' ATTGTGCTGCATTCAACCAAACTGATCCAAGAGTGGAACTCTGTGGACCTTAGGACCCCCCTTTCTGACACTTTGCATCT
4400
GNE ORF
Translation 2872-5040
5' CCCTGTGTGGGTAGACAATGATGGCAACTGTGCTGCCCTGGCGGAAAGGAAATTTGGCCAAGGAAAGGGACTGGAAAACT
4480
GNE ORF
Translation 2872-5040
5' TTGTTACACTTATCACAGGCACAGGAATCGGTGGTGGAATTATCCATCAGCATGAATTGATCCACGGAAGCTCCTTCTGT
4560
GNE ORF
Translation 2872-5040

FIG. 2 (cont.)

pAAV.CMV.GNE.spA.miniCM

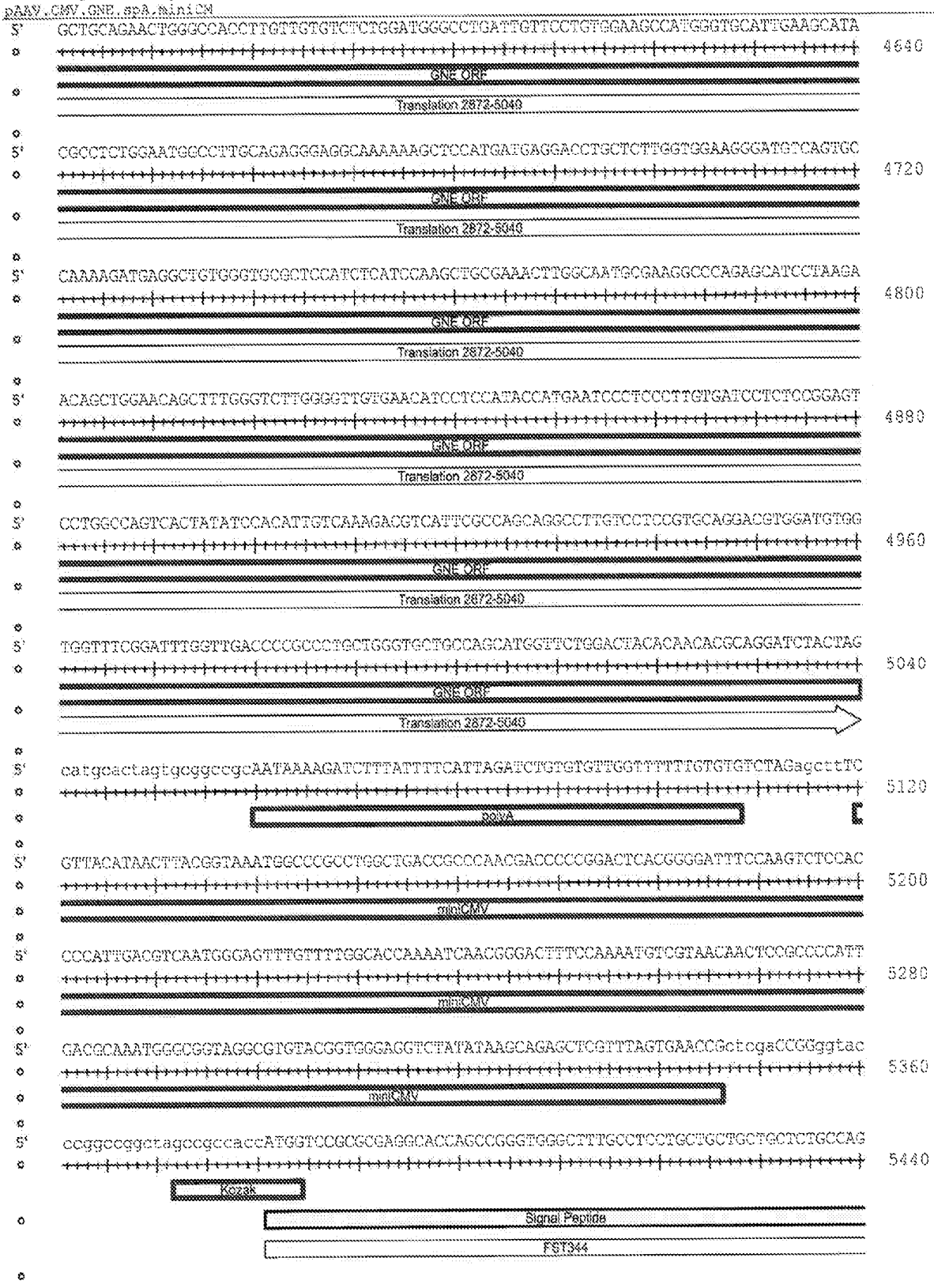


FIG. 2 (cont.)

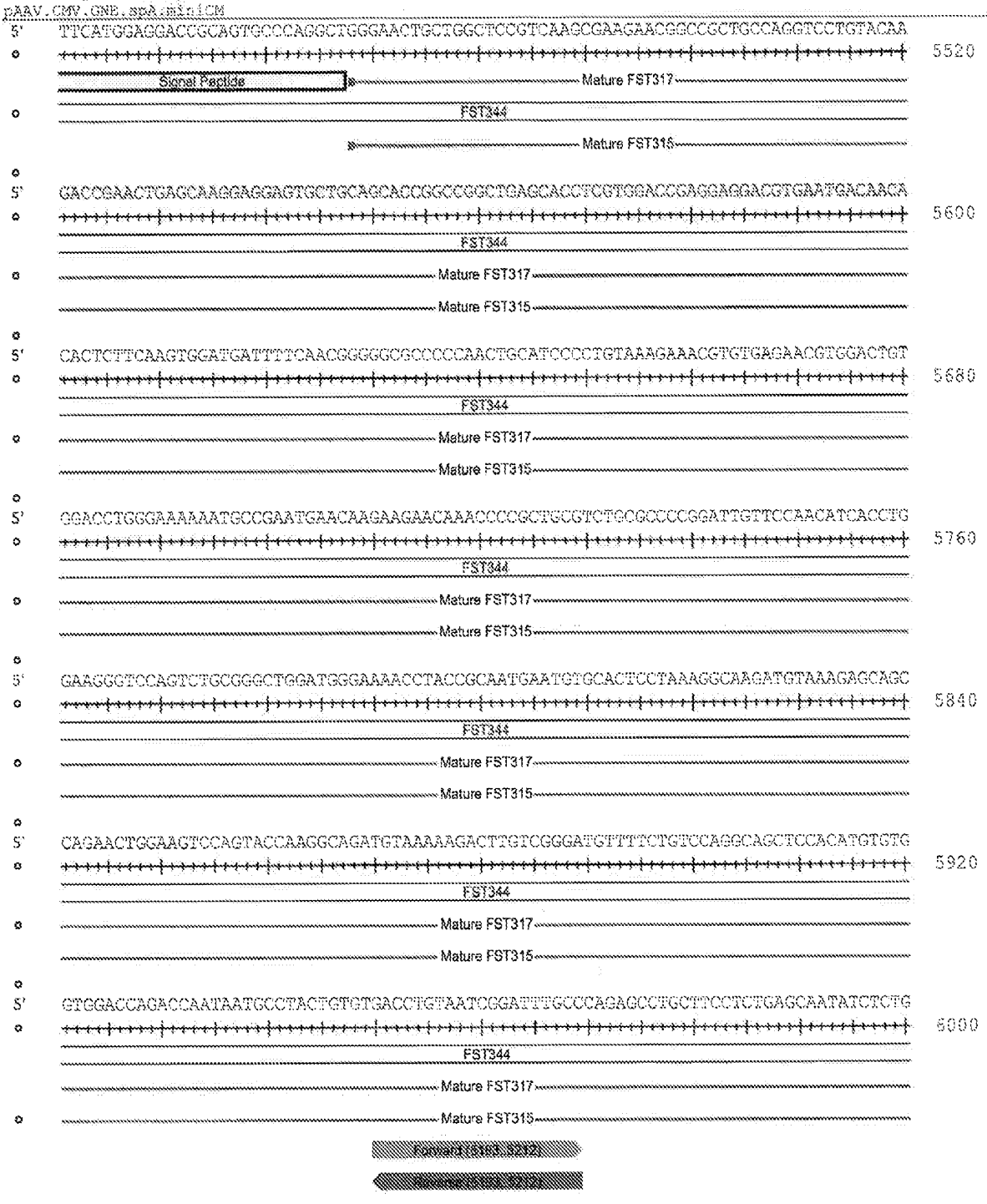

5' TTCATGGAGGACCGCAGTGCCCAGGCTGGGAACTGCTGGCTCCGTCAAGCGAAGAACGGCCGCTGCCAGGTCCTGTACAA
5520
Signal Peptide
Mature FST317
FST344
Mature FST315
5' GACCGAACTGAGCAAGGAGGAGTGCTGCAGCACCGGCCGGCTGAGCACCTCGTGGACCGAGGAGGACGTGAATGACAACA
5600
FST344
Mature FST317
Mature FST315
5' CACTCTTCAAGTGGATGATTTTCAACGGGGGCGCCCCCAACTGCATCCCCTGTAAAGAAACGTGTGAGAACGTGGACTGT
5680
FST344
Mature FST317
Mature FST315
5' GGACCTGGGAAAAAATGCCGAATGAACAAGAAGAACAAACCCCGCTGCGTCTGCGCCCCGGATTGTTCCAACATCACCTG
5760
FST344
Mature FST317
Mature FST315
5' GAAGGGTCCAGTCTGCGGGCTGGATGGGAAAACCTACCGCAATGAATGTGCACTCCTAAAGGCAAGATGTAAAGAGCAGC
5840
FST344
Mature FST317
Mature FST315
5' CAGAACTGGAAGTCCAGTACCAAGGCAGATGTAAAAAGACTTGTCGGGATGTTTTCTGTCCAGGCAGCTCCACATGTGTG
5920
FST344
Mature FST317
Mature FST315
5' GTGGACCAGACCAATAATGCCTACTGTGTGACCTGTAATCGGATTTGCCCAGAGCCTGCTTCCTCTGAGCAATATCTCTG
6000
FST344
Mature FST317
Mature FST315
Forward (5183..5212)
Reverse (5183..5212)

FIG. 2 (cont.)

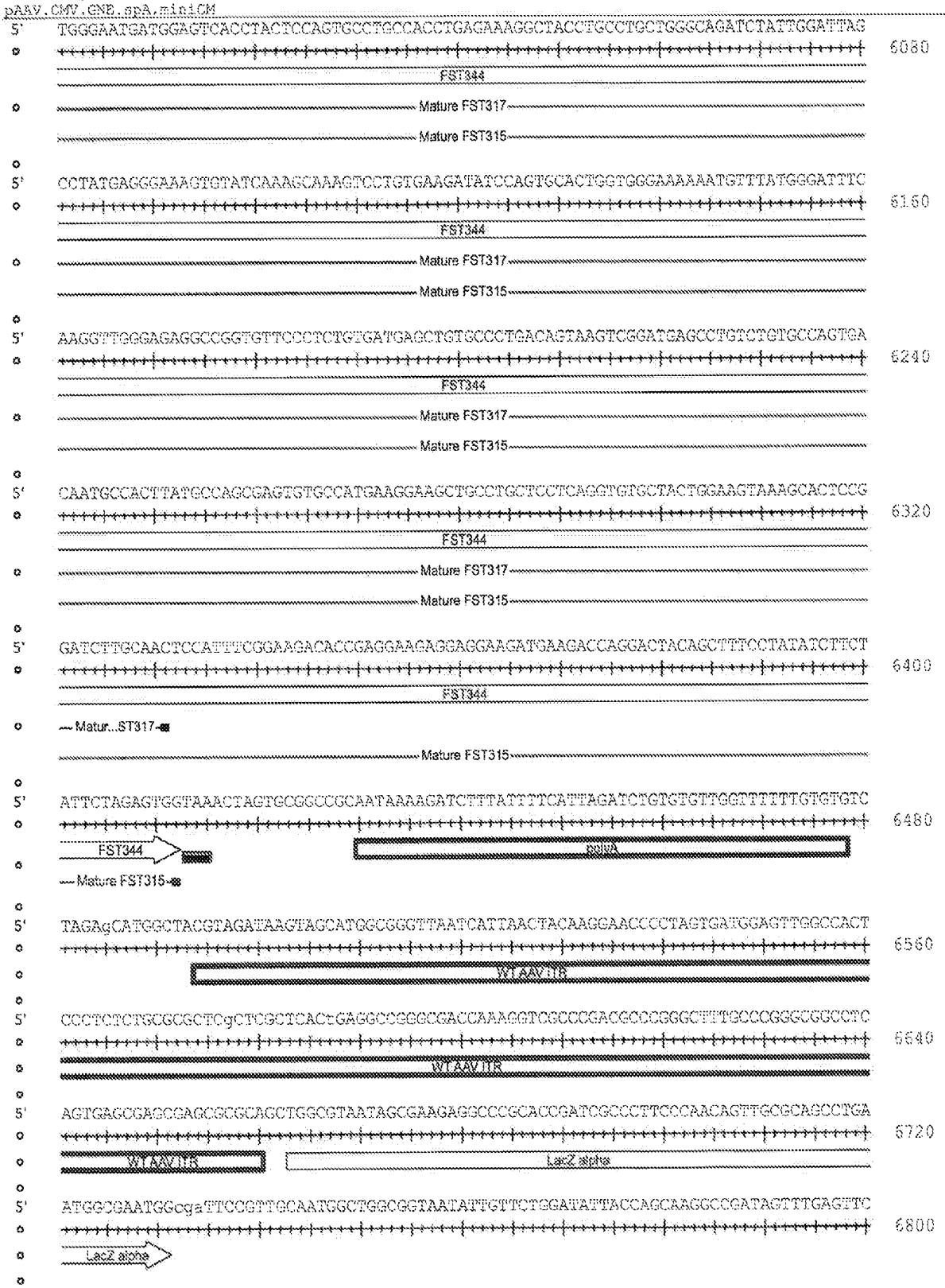

5' TGGGAATGATGGAGTCACCTACTCCAGTGCCTGCCACCTGAGAAAGGCTACCTGCCTGCTGGGCAGATCTATTGGATTAG
6080
FST344
Mature FST317
Mature FST315
5' CCTATGAGGGAAAGTGTATCAAAGCAAAGTCCTGTGAAGATATCCAGTGCACTGGTGGGAAAAAATGTTTATGGGATTTC
6160
FST344
Mature FST317
Mature FST315
5' AAGGTTGGGAGAGGCCGGTGTTCCCTCTGTGATGAGCTGTGCCCTGACAGTAAGTCGGATGAGCCTGTCTGTGCCAGTGA
6240
FST344
Mature FST317
Mature FST315
5' CAATGCCACTTATGCCAGCGAGTGTGCCATGAAGGAAGCTGCCTGCTCCTCAGGTGTGCTACTGGAAGTAAAGCACTCCG
6320
FST344
Mature FST317
Mature FST315
5' GATCTTGCAACTCCATTTCCGAAGACACCGAGGAAGAGGAGGAAGATGAAGACCAGGACTACAGCTTTCCTATATCTTCT
6400
FST344
Matur...ST317
Mature FST315
5' ATTCTAGAGTGGTAAACTAGTGCGGCCGCAATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGTTTTTTGTGTGTC
6480
FST344
polyA
Mature FST315
5' TAGAgCATGGCTACGTAGATAAGTAGCATGGCGGGTTAATCATTAACTACAAGGAACCCCTAGTGATGGAGTTGGCCACT
6560
WT AAV ITR
5' CCCTCTCTGCGCGCTCgCTCGCTCACtGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTC
6640
WT AAV ITR
5' AGTGAGCGAGCGAGCGCGCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGA
6720
WT AAV ITR
Lac2 alpha
5' ATGGCGAATGGcgaTTCCGTTGCAATGGCTGGCGGTAATATTGTTCTGGATATTACCAGCAAGGCCGATAGTTTGAGTTC
6800
Lac2 alpha

FIG. 2 (cont.)

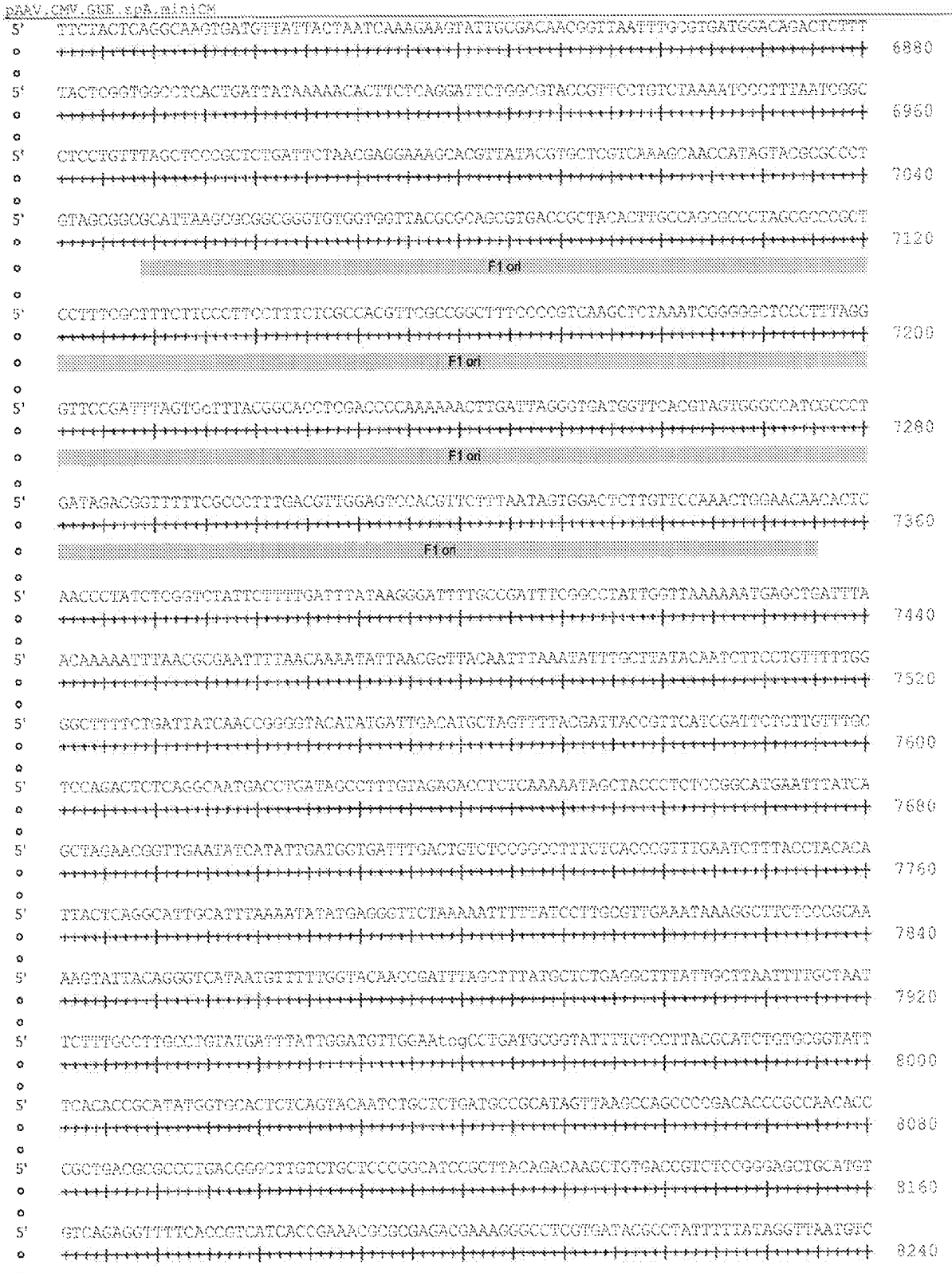

5' TTCTACTCAGGCAAGTGATGTTATTACTAATCAAAGAAGTATTGCGACAACGGTTAATTTGCGTGATGGACAGACTCTTT
6880
5' TACTCGGTGGCCTCACTGATTATAAAAACACTTCTCAGGATTCTGGCGTACCGTTCCTGTCTAAAATCCCTTTAATCGGC
6960
5' CTCCTGTTTAGCTCCCGCTCTGATTCTAACGAGGAAAGCACGTTATACGTGCTCGTCAAAGCAACCATAGTACGCGCCCT
7040
5' GTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCT
7120
F1 ori
5' CCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGG
7200
F1 ori
5' GTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCT
7280
F1 ori
5' GATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTC
7360
F1 ori
5' AACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTA
7440
5' ACAAAAATTTAACGCGAATTTTAACAAAATATTAACGcTTACAATTTAAATATTTGCTTATACAATCTTCCTGTTTTTGG
7520
5' GGCTTTTCTGATTATCAACCGGGGTACATATGATTGACATGCTAGTTTTACGATTACCGTTCATCGATTCTCTTGTTTGC
7600
5' TCCAGACTCTCAGGCAATGACCTGATAGCCTTTGTAGAGACCTCTCAAAAATAGCTACCCTCTCCGGCATGAATTTATCA
7680
5' GCTAGAACGGTTGAATATCATATTGATGGTGATTTGACTGTCTCCGGCCTTTCTCACCCGTTTGAATCTTTACCTACACA
7760
5' TTACTCAGGCATTGCATTTAAAATATATGAGGGTTCTAAAAATTTTTATCCTTGCGTTGAAATAAAGGCTTCTCCCGCAA
7840
5' AAGTATTACAGGGTCATAATGTTTTTGGTACAACCGATTTAGCTTTATGCTCTGAGGCTTTATTGCTTAATTTTGCTAAT
7920
5' TCTTTGCCTTGCCTGTATGATTTATTGGATGTTGGAAtagCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATT
8000
5' TCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACC
8080
5' CGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGT
8160
5' GTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTC
8240

FIG. 2 (cont.)

```
5'   ATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTA
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  8320
o
5'   AATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGA
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  8400
o
5'   GTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTG
o    ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|  8480
o
5'   GTGAAAGTAAAAGATGCTGAAGATCA
o    ++++|++++|++++|++++|++++|++  8506
o
```

FIG. 2 (cont.)

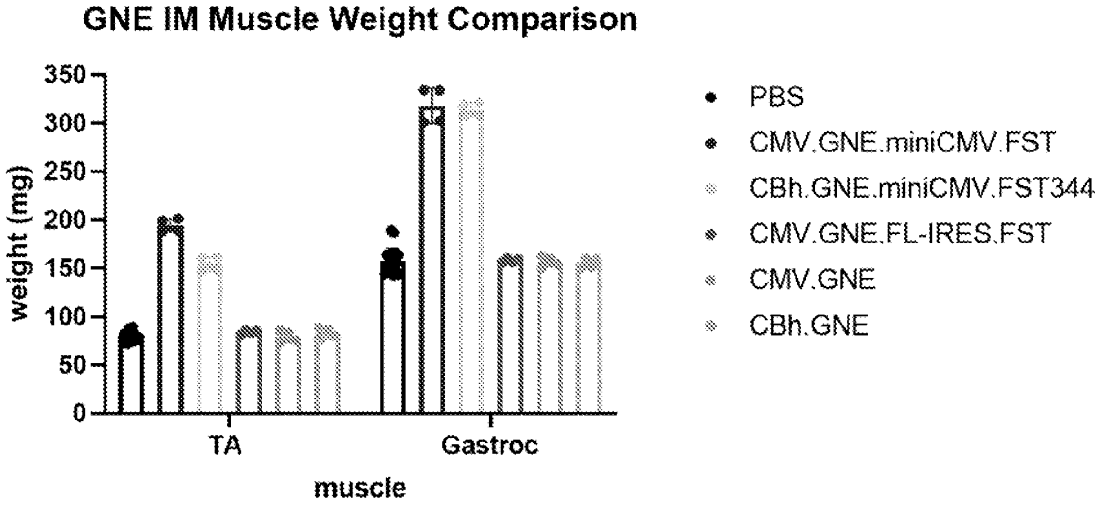

GNE IM Muscle Weight Comparison
weight (mg)
350
300
250
200
150
100
50
0
TA
Gastroc
muscle
PBS
CMV.GNE.miniCMV.FST
CBh.GNE.miniCMV.FST344
CMV.GNE.FL-IRES.FST
CMV.GNE
CBh.GNE

FIG. 3

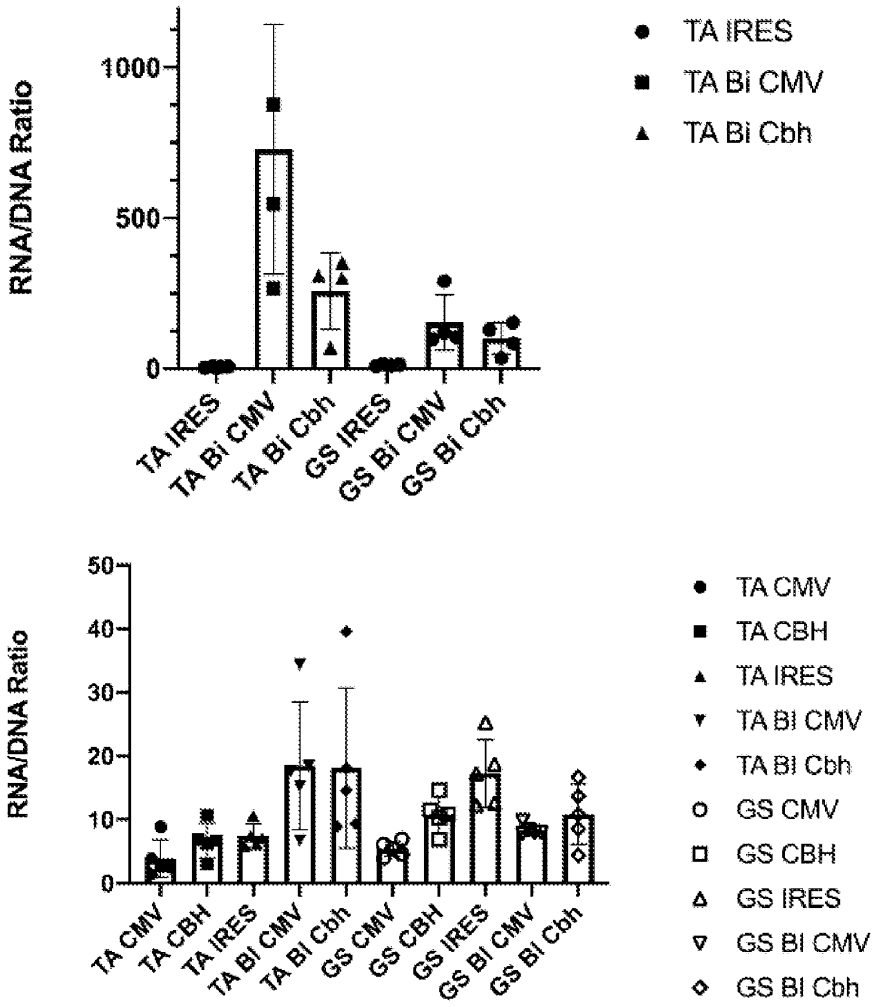

RNA/DNA Ratio
1000
500
0
TA IRES
TA Bi CMV
TA Bi Cbh
GS IRES
GS Bi CMV
GS Bi Cbh
RNA/DNA Ratio
50
40
30
20
10
0
TA CMV
TA CBH
TA IRES
TA Bi CMV
TA Bi Cbh
GS CMV
GS CBH
GS IRES
GS Bi CMV
GS Bi Cbh

FIG. 4

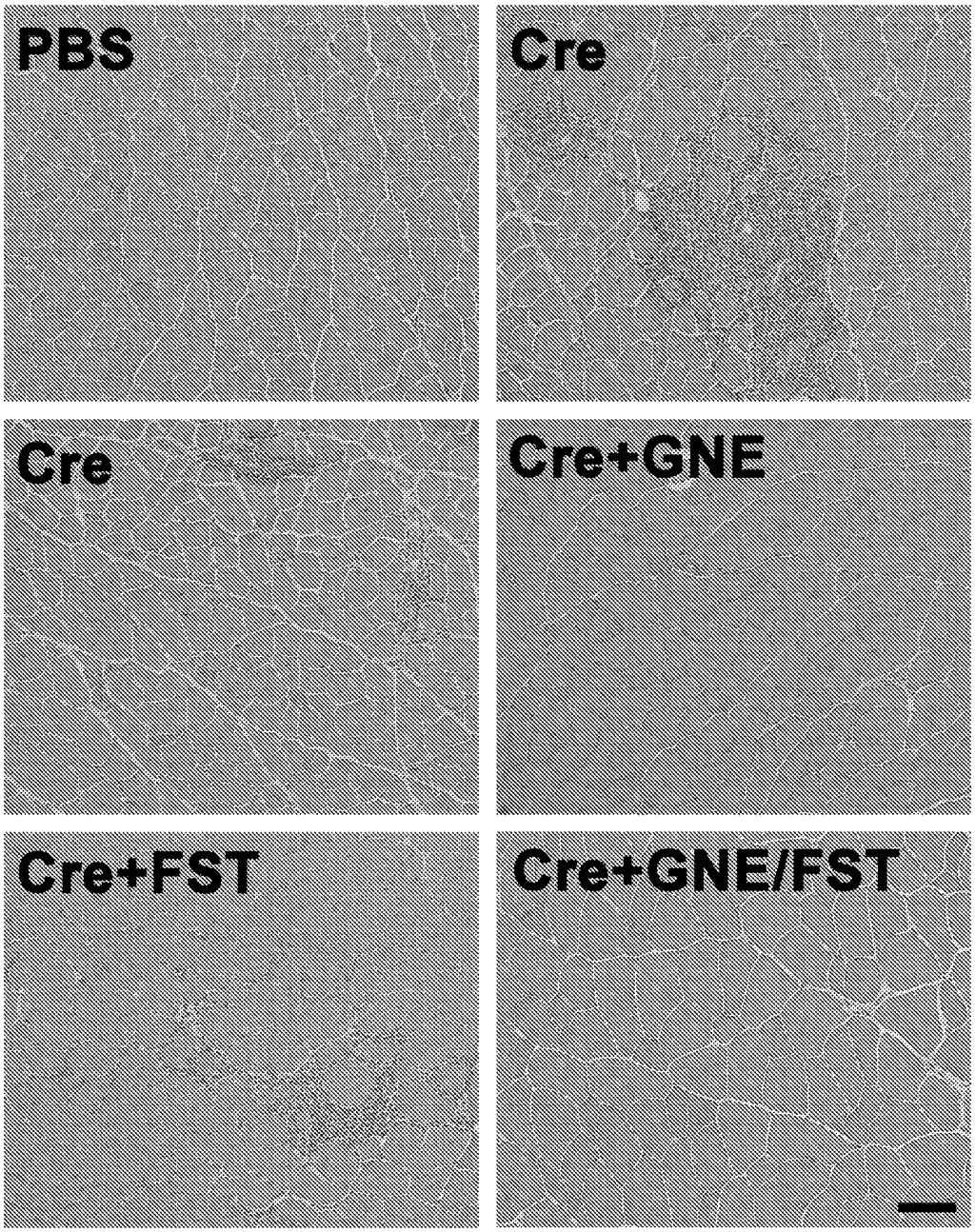


FIG. 5

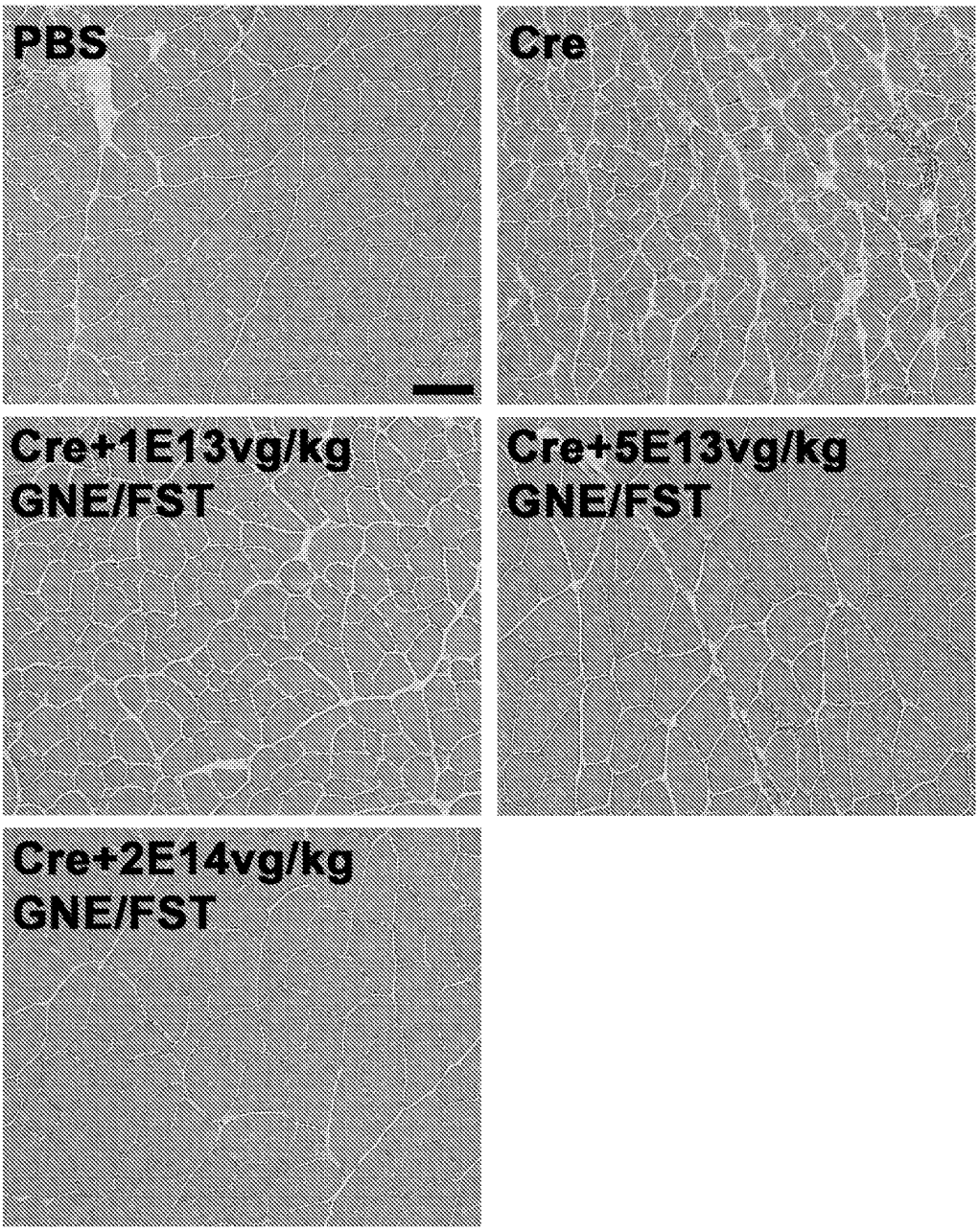

PBS
Cre
Cre+1E13vg/kg
GNE/FST
Cre+5E13vg/kg
GNE/FST
Cre+2E14vg/kg
GNE/FST

FIG. 6

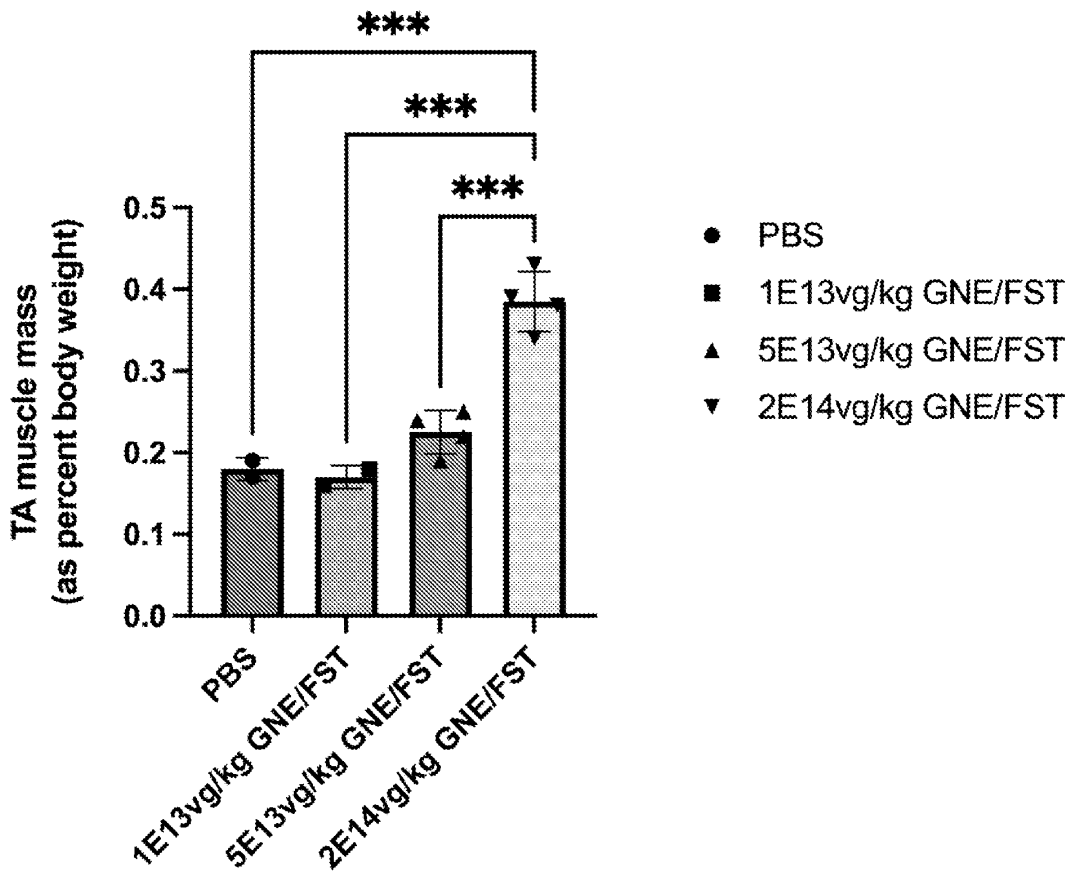

***
***
***
TA muscle mass
(as percent body weight)
0.5
0.4
0.3
0.2
0.1
0.0
PBS
1E13vg/kg GNE/FST
5E13vg/kg GNE/FST
2E14vg/kg GNE/FST
PBS
1E13vg/kg GNE/FST
5E13vg/kg GNE/FST
2E14vg/kg GNE/FST

FIG. 7

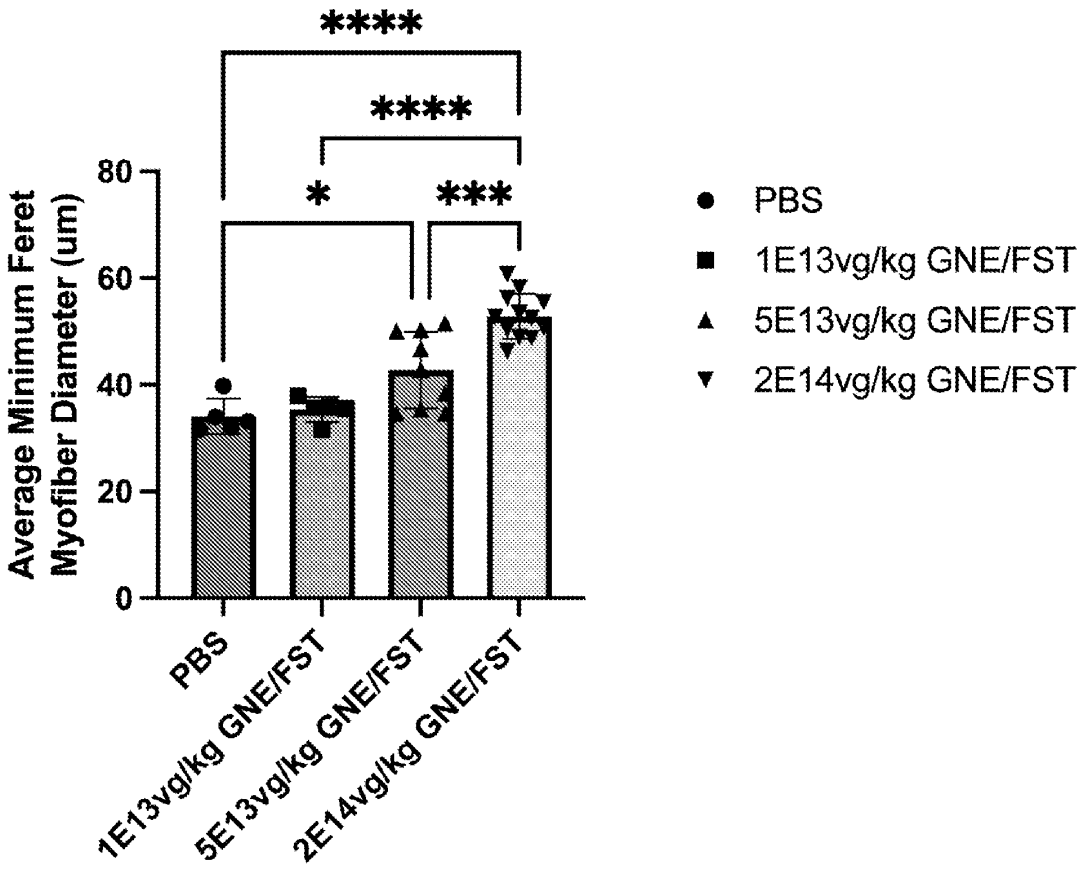

****
****
*
***
Average Minimum Feret Myofiber Diameter (um)
0
20
40
60
80
PBS
1E13vg/kg GNE/FST
5E13vg/kg GNE/FST
2E14vg/kg GNE/FST
PBS
1E13vg/kg GNE/FST
5E13vg/kg GNE/FST
2E14vg/kg GNE/FST

FIG. 8