An Improved SNAP-ADAR Tool Enables Efficient RNA Base Editing to Interfere with Post-translational Protein Modification

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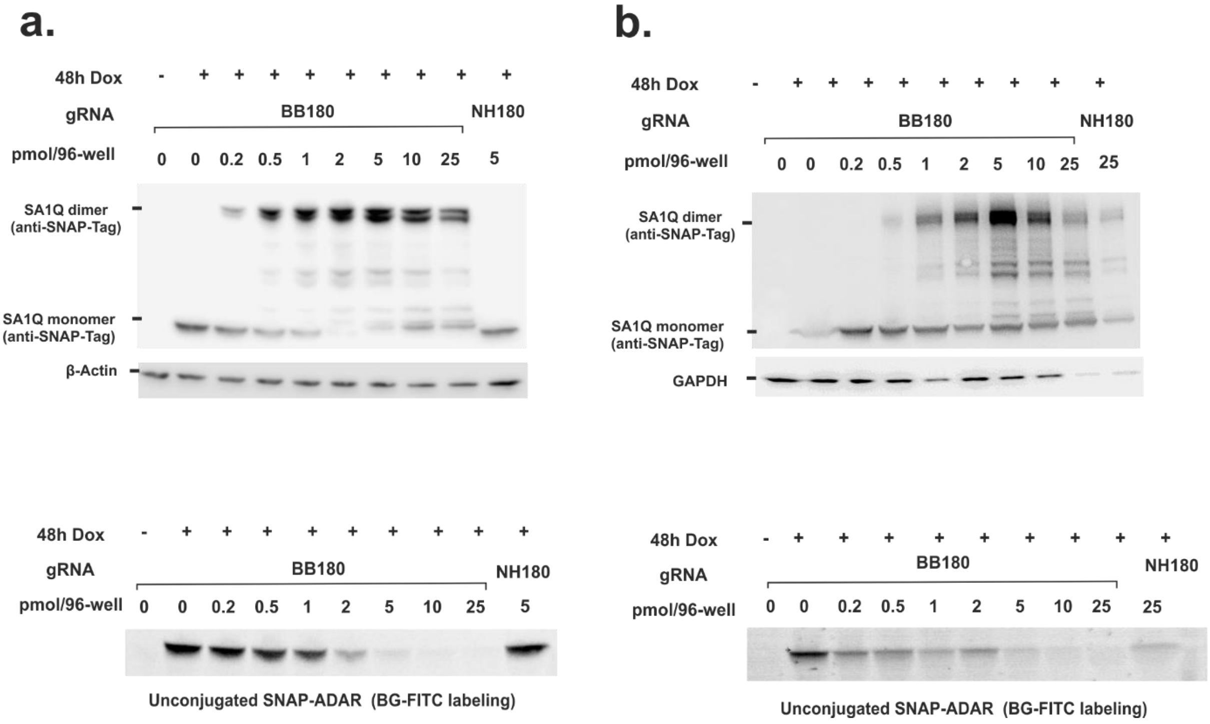
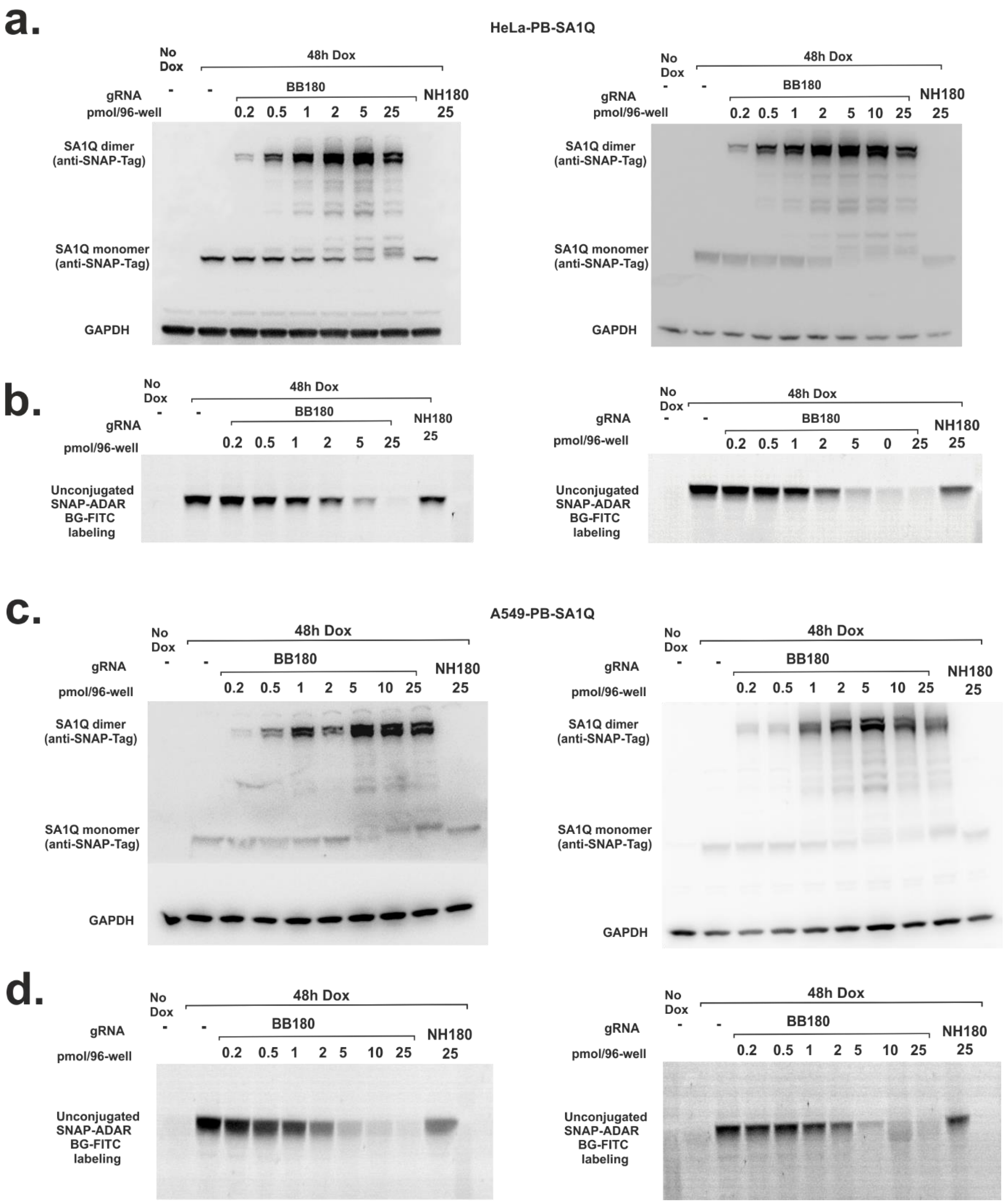
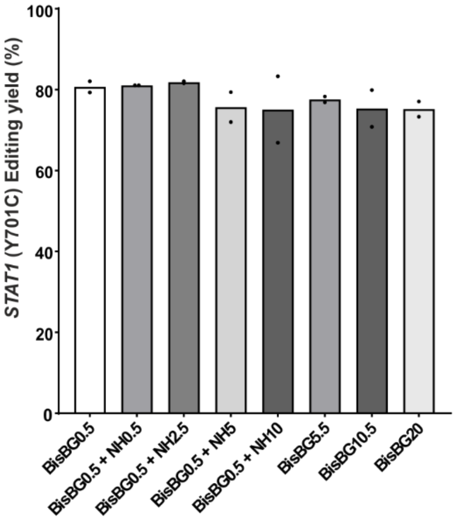
Supplementary Figures



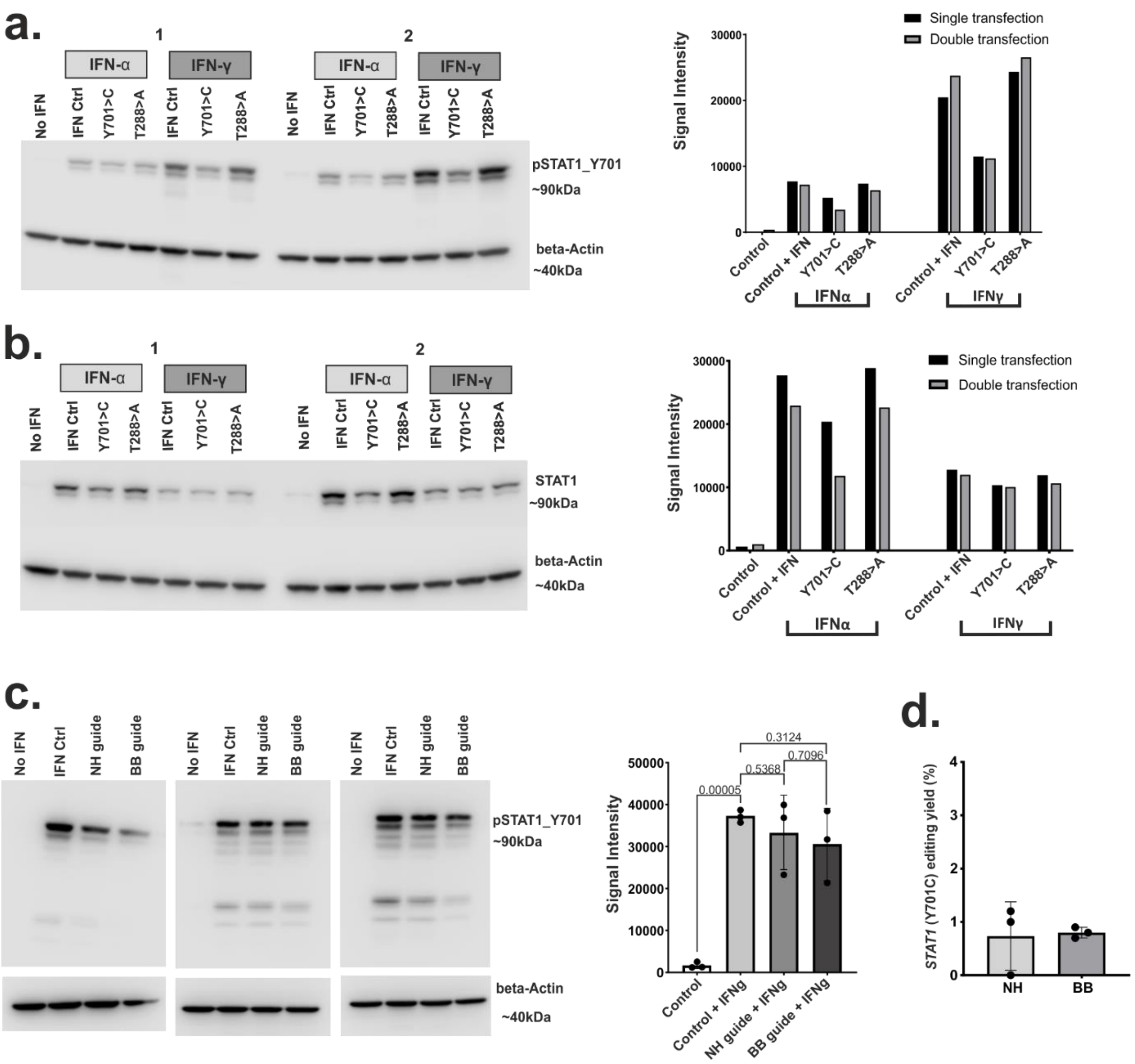
Supplementary Figure 1 Blots showing guideRNA dose-dependent dimerization of the effector SA1Q in Flp-In T-Rex 293 – SA1Q cells induced with 10ng/ml Doxycycline for 48 hrs. Cells were transfected with indicated amounts of BB180 (BisBG-25 nt + LNA gRNA against STAT1 Y701 site) or NH180 (gRNA lacking the BisBG moiety for SA attachment). **A)** and **B)** are biological replicates (N=2) of Fig.1d, with Western blot (blot on top) and BG-FITC -protein/gRNA conjugation assay blot (blot in the bottom) respectively, showing gRNA dose-dependent of SA1Q dimerization in cells induced for 48hrs with Doxycycline. GAPDH/ß-Actin served as loading controls. All guide RNA amounts denote pmol/96-well with 150 µl total volume. Western blotting and BG-FITC staining were done as described in the methods section. Full blots are available in the Source Data file.



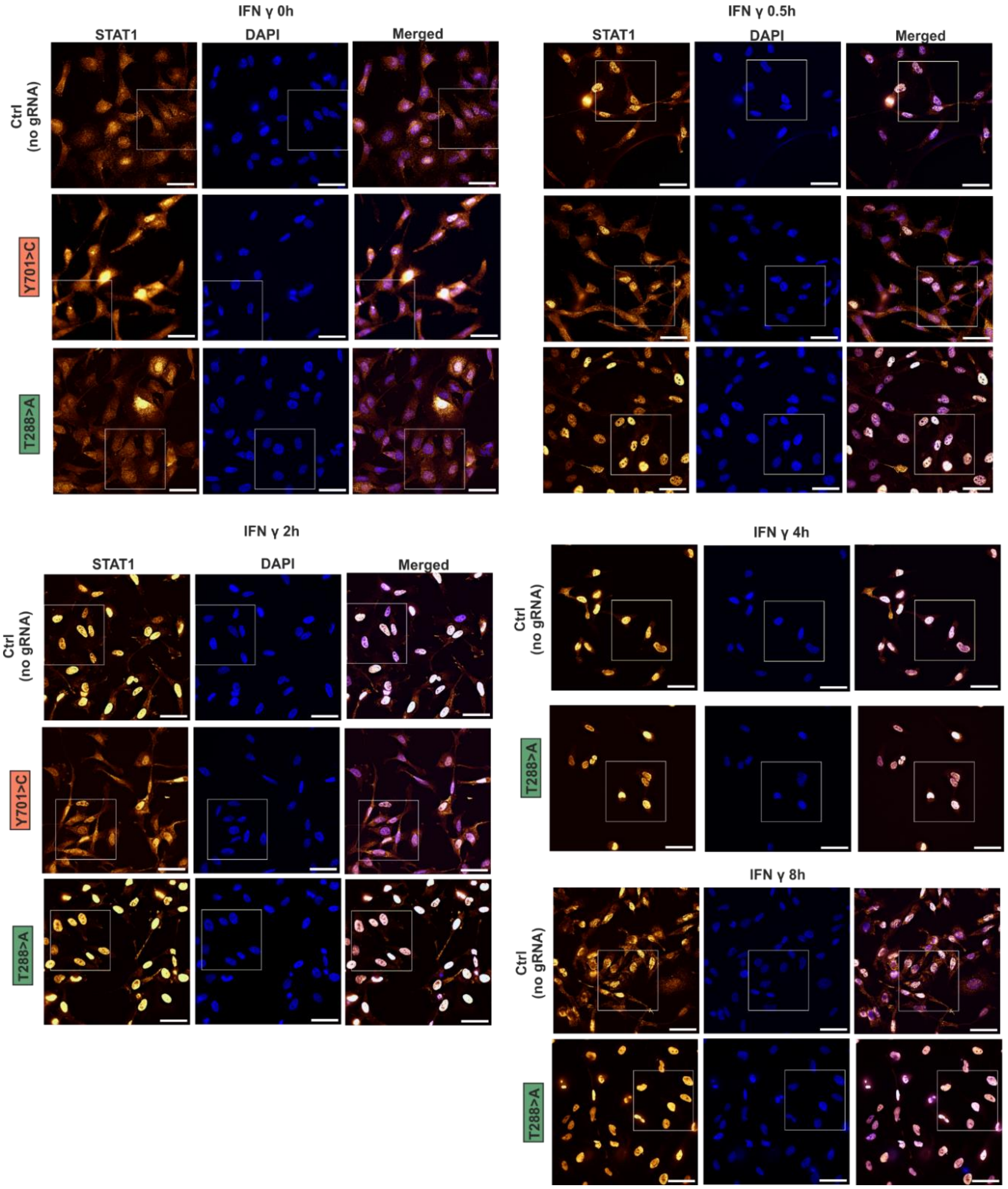
Supplementary Figure 2. Blots showing biological replicates (N=2) of SNAP-ADAR protein expression and its conjugation with gRNA studied in Hela-PB-SA1Q and A549-PB-SA1Q cells using Western blot and BG-FITC -protein/gRNA conjugation assay. Cells were transfected with different amounts of BisBG-25 nt + LNA gRNA against STAT1 Y701 site (BB180) in 96-well plate set up with 1µg/ml doxycycline induction for 48h. NH180 denotes gRNA lacking the BisBG moiety for SA attachment. **A)** and **B)** show Western blots and BG-FITC blots (replicates) respectively for Hela-PB-SA1Q cells. **C)** and **D)** show Western blots and BG-FITC blots (replicates) respectively for A549-PB-SA1Q cells. GAPDH served as loading control. All guide RNA amounts denote pmol/96-well with 150 µl total volume. Western blotting and BG-FITC staining were done as described in the methods section. Full blots are available in the Source Data file.



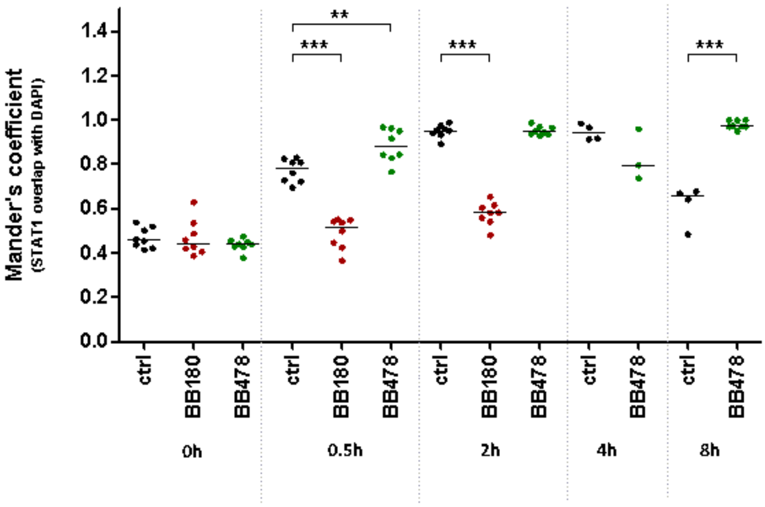
Supplementary Figure 3. Competitive editing experiment of editing-competent BisBG guide RNAs with editing-incompetent amino guide RNA of the same sequence and chemical modification. In Flp-In T-REx 293 SA1Q cells, the editing-competent BisBG 180 guide (0.5 pmol per 96 well) and an increasing amount (0.5-10 pmol per 96-well) of the same editing-incompetent NH180 control guide RNA against the transcript STAT1 (Y701>C site) were co-transfected. Even a 20x excess of amino control guide RNA (NH180) did not reduce the editing yield with 0.5 pmol BisBG guide RNA, indicating that the guide RNAs act on the target RNA rather in a hit-and-run fashion than staying at the target for a long time. Furthermore, editing yield for increasing BisBG guide RNA concentrations (up to 20 pmol/96 well) are shown. The data is presented as N=2 biological replicates, as indicated by individual data points. Source data are provided in Source Data file.



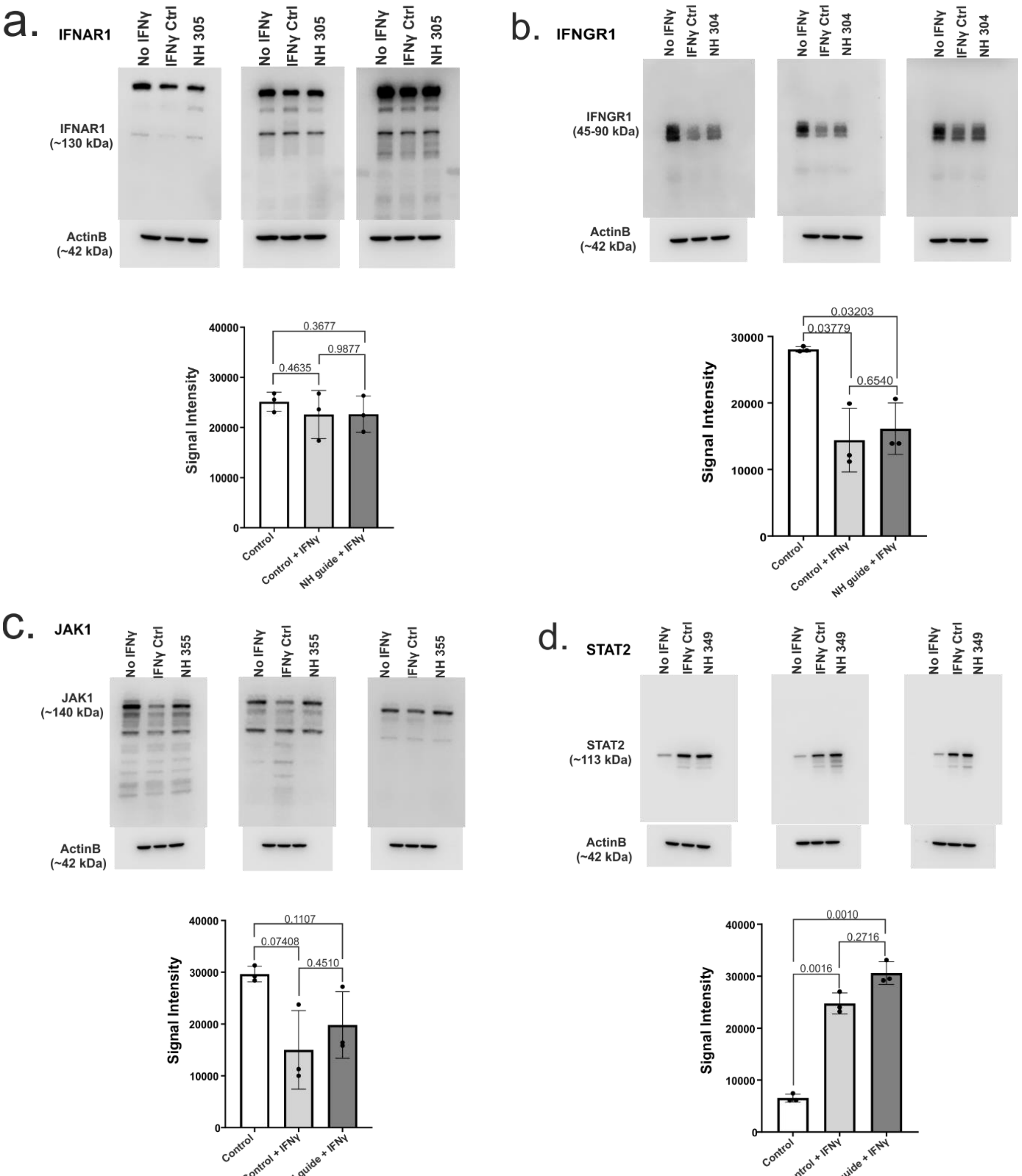
Supplementary Figure 4. Analysis of STAT1 activation (tyrosine 701 phosphorylation) by Western Blot in response to up (T288>A) and down regulating (Y701>C) PTMi. **A)** Shows phospho-STAT1 and **B)** shows total STAT1 in response to various PTMi settings and induction by interferon-α and -γ, as indicated. Experiment **1** in **A)** and **B):** Dox (1 µg/ml, 72h total), single transfection (T1) in 24 well plate with 2x10^5 cells and 8 pmol guide per well, 2000 u/ml IFN α or γ induction 24h prior to lysis. Experiment **2** in **A)** and **B):** Dox (1 µg/ml, 120h total), double transfection (T2) with 48h in between, in 24 well plate with $2x10^5$ cells and 8 pmol guide per well, 2000 U/ml IFN-α or -γ induction 24h prior to lysis. **C)** Effect of an editing-incompetent guide RNA that can (BB482 = BisBG 482) or cannot (NH482) recruit the SNAP-ADAR effector on target protein expression (pSTAT1) by Western Blot. The bar graph includes a statistical analysis of the band intensities, with P values indicated. Applied was a 2-winged t-test, N=3; P>0.05 is interpreted as non-significant. **D)** The editing-incompetent guide RNA BisBG 482 was unable to induce the Y701>C edit in STAT1. Source data are provided as a Source Data file.



Supplementary Figure 5. Immunofluorescence imaging of total STAT1 cellular localization upon different gRNA treatment vs control (no gRNA), at various time points post IFN-γ induction. Insets show zoomed-in areas shown in Fig. 5d. For detailed protocol and list of antibodies, see the Methods section and Supplementary Table 1, respectively. Images were taken with a Nikon Eclipse Ti2-E inverted fluorescent microscope, equipped with a photometrics® Prime 95B camera and a lumencor® AuraII light engine. All pictures were recorded using a 60x oil objective (numerical aperture 1.4) and Olympus IMMOIL-F30CC immersion oil. The excitation wavelengths and corresponding filter sets used to record each channel are specified in Supplementary Table 2. The same acquisition settings were chosen for each channel for all images. The images were 3D deconvoluted, and further processed in FIJI ImageJ[1]. Amber LUT, used to display STAT1 protein, was obtained from ImageJ Wiki – NucMed LUT list [2].For more details on LUT, see Source Data.



Supplementary Figure 6. Colocalization analysis. Mander's coefficient for the overlap of total STAT1 protein signal with nuclear DAPI signal was determined and plotted for each condition using the JACoP plug-in[3] in FIJI ImageJ[1]. An unbiased sectioning of biological replicate images (N =2 for most, N =1 for some) into quadrants was done. Significance for N= 3,4 or 8 sections was determined by an unpaired, two-tailed t-test, $P \leq 0.05$ = *, $P \leq 0.01$ = **, $P \leq 0.001$ = ***, $P>0.05$ = nonsignificant. Individual data points are given, the median is denoted by a black line. Source data are provided as a Source Data file.



Supplementary Figure 7. Analysis of the effect of guide RNA transfection with or without IFN-γ induction on the protein expression of four different PTMi targets by Western Blot. The bar graphs show the statistical analysis of the band intensities, with P values indicated. Applied was a 2-winged t-test, N=3; P>0.05 is interpreted as non-significant. **A)** For the IFNAR1 target. **B)** For the IFNGR1 target. **C)** For the JAK1 target. **D)** For the STAT2 target. Dox (1 µg/ml, 120h total), double transfection (T2) with 48 h in between, in 24 well plate with $2x10^5$ cells and 8 pmol guide per well, 2000 u/ml IFN-γ induction 24 h prior to cell lysis. Full Western blots and Source data are provided as a Source Data file.

References

1. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* **9**, 671–675 (2012).
2. https://imagej.net/ij/download/luts/NucMed_Image_LUTs/.
3. BOLTE, S. & CORDELIÈRES, F. P. A guided tour into subcellular colocalization analysis in light microscopy. *J Microsc* **224**, 213–232 (2006).

Supplementary Tables

Supplementary Table 1 All antibodies were diluted in 5% dry milk in 1x TBST buffer.

Antibody	**Cat.no.**	**Manufacturer**	**Dilution**
Mouse α-Beta-Actin	A5441	Sigma	1: 5000
Rabbit α-SNAP-tag	P9310S	New England Biolabs	1: 1000
α-SNAP Tag Polyclonal Antibody	CAB4255	Thermo-Fisher Scientific	1: 1000
Mouse-α-GAPDH Loading Control Monoclonal Antibody (GA1R)	MA5-15738	Thermo-Fisher Scientific	1: 1000
Mouse-α-GAPDH Monoclonal Antibody (6C5)	AM4300	Thermo-Fisher Scientific	1: 1000
Rabbit-α-Gapdh (D16H11) XP	5174T/5174S	Cell Signalling	1: 2000
pSTAT1(Y701)	9167	Cell Signalling	1: 1000
STAT1 mouse	9176	Cell Signalling	1: 1000
STAT1 (42H3) Rabbit	9175	Cell Signalling	1: 1000
Goat α-Rabbit Alexa Fluor 594	8889S	Cell Signalling	1: 1000
Goat α-Rabbit HRP	111-035-003	Jackson Immuno Research Laboratories	1: 10000
Goat α-Mouse HRP	115-035-003	Jackson Immuno Research Laboratories	1: 10000
IFNAR1 rabbit	MA5-32006	Thermo-Fisher Scientific	1: 1000
JAK1 mouse	50996	Cell Signaling	1: 1000
STAT1 Rabbit	9172	Cell Signaling	1: 1000
IFNGR1 rabbit	34808	Cell Signaling	1: 1000
STAT2 rabbit	72604	Cell Signaling	1: 1000

Supplementary Table 2 Excitation wavelengths and corresponding filter sets used with Nikon Eclipse Ti2-Emicroscope.

Fluorophore	**Excitation**	**Filter**
eGFP/FITC	475 nM	Excitation: Chroma 89402x Beamsplitter: Chroma 89402bs Emission: Chroma 89402m
Atto594	575 nM	Excitation: Semrock 585/29 BrightLine HC Beamsplitter: Chroma T610LPXR Emission: Semrock 650/60 BrightLine HC
NucBlue (DAPI)	390 nM	Excitation: Chroma 89402x Beamsplitter: Chroma 89402bs Emission: Chroma 89402m

Gene and protein sequences for SNAP-ADAR versions used:

Gene & protein sequence of the SA1Q as expressed from engineered Flp-In-TREx 293 genome. E>Q site is highlighted in cyan.

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                  10        20        30        40        50        60
1         ATGGACAAAGACTGCGAAATGAAGCGCACCACCCTGGATAGCCCTCTGGGCAAGCTGGAA
1          M  D  K  D  C  E  M  K  R  T  T  L  D  S  P  L  G  K  L  E

                  70        80        90       100       110       120
61        CTGTCTGGGTGCGAACAGGGCCTGCACCGTATCATCTTCCTGGGCAAAGGAACATCTGCC
21         L  S  G  C  E  Q  G  L  H  R  I  I  F  L  G  K  G  T  S  A

                 130       140       150       160       170       180
121       GCCGACGCCGTGGAAGTGCCTGCCCCAGCCGCCGTGCTGGGCGGACCAGAGCCACTGATG
41         A  D  A  V  E  V  P  A  P  A  A  V  L  G  G  P  E  P  L  M

                 190       200       210       220       230       240
181       CAGGCCACCGCCTGGCTCAACGCCTACTTTCACCAGCCTGAGGCCATCGAGGAGTTCCCT
61         Q  A  T  A  W  L  N  A  Y  F  H  Q  P  E  A  I  E  E  F  P

                 250       260       270       280       290       300
241       GTGCCAGCCCTGCACCACCCAGTGTTCCAGCAGGAGAGCTTTACCCGCCAGGTGCTGTGG
81         V  P  A  L  H  H  P  V  F  Q  Q  E  S  F  T  R  Q  V  L  W

                 310       320       330       340       350       360
301       AAACTGCTGAAAGTGGTGAAGTTCGGAGAGGTCATCAGCTACAGCCACCTGGCCGCCCTG
101        K  L  L  K  V  V  K  F  G  E  V  I  S  Y  S  H  L  A  A  L

                 370       380       390       400       410       420
361       GCCGGCAATCCCGCCGCCACCGCCGCCGTGAAAACCGCCCTGAGCGGAAATCCCGTGCCC
121        A  G  N  P  A  A  T  A  A  V  K  T  A  L  S  G  N  P  V  P

                 430       440       450       460       470       480
421       ATTCTGATCCCCTGCCACCGGGTGGTGCAGGGCGACCTGGACGTGGGGGGCTACGAGGGC
141        I  L  I  P  C  H  R  V  V  Q  G  D  L  D  V  G  G  Y  E  G

                 490       500       510       520       530       540
481       GGGCTCGCCGTGAAAGAGTGGCTGCTGGCCCACGAGGGCCACAGACTGGGCAAGCCTGGG
161        G  L  A  V  K  E  W  L  L  A  H  E  G  H  R  L  G  K  P  G

                 550       560       570       580       590       600
541       CTGGGTCCTGCAGGCGGAGGCGCGCCAGGGTCTGGCGGCGGCAGTAAGGCAGAACGCATG
181        L  G  P  A  G  G  G  A  P  G  S  G  G  G  S  K  A  E  R  M

                 610       620       630       640       650       660
601       GGTTTCACAGAGGTAACCCCAGTGACAGGGGCCAGTCTCAGAAGAACTATGCTCCTCCTC
201        G  F  T  E  V  T  P  V  T  G  A  S  L  R  R  T  M  L  L  L

                 670       680       690       700       710       720
661       TCAAGGTCCCCAGAAGCACAGCCAAAGACACTCCCTCTCACTGGCAGCACCTTCCATGAC
221        S  R  S  P  E  A  Q  P  K  T  L  P  L  T  G  S  T  F  H  D

                 730       740       750       760       770       780
721       CAGATAGCCATGCTGAGCCACCGGTGCTTCAACACTCTGACTAACAGCTTCCAGCCCTCC
241        Q  I  A  M  L  S  H  R  C  F  N  T  L  T  N  S  F  Q  P  S
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                 790       800       810       820       830       840
781      TTGCTCGGCCGCAAGATTCTGGCCGCCATCATTATGAAAAAAGACTCTGAGGACATGGGT
261       L  L  G  R  K  I  L  A  A  I  I  M  K  K  D  S  E  D  M  G

                 850       860       870       880       890       900
841      GTCGTCGTCAGCTTGGGAACAGGGAATCGCTGTGTAAAAGGAGATTCTCTCAGCCTAAAA
281       V  V  V  S  L  G  T  G  N  R  C  V  K  G  D  S  L  S  L  K

                 910       920       930       940       950       960
901      GGAGAAACTGTCAATGACTGCCATGCAGAAATAATCTCCCGGAGAGGCTTCATCAGGTTT
301       G  E  T  V  N  D  C  H  A  E  I  I  S  R  R  G  F  I  R  F

                 970       980       990      1000      1010      1020
961      CTCTACAGTGAGTTAATGAAATACAACTCCCAGACTGCGAAGGATAGTATATTTGAACCT
321       L  Y  S  E  L  M  K  Y  N  S  Q  T  A  K  D  S  I  F  E  P

                1030      1040      1050      1060      1070      1080
1021     GCTAAGGGAGGAGAAAAGCTCCAAATAAAAAAGACTGTGTCATTCCATCTGTATATCAGC
341       A  K  G  G  E  K  L  Q  I  K  K  T  V  S  F  H  L  Y  I  S

                1090      1100      1110      1120      1130      1140
1081     ACTGCTCCGTGTGGAGATGGCGCCCTCTTTGACAAGTCCTGCAGCGACCGTGCTATGGAA
361       T  A  P  C  G  D  G  A  L  F  D  K  S  C  S  D  R  A  M  E

                1150      1160      1170      1180      1190      1200
1141     AGCACAGAATCCCGCCACTACCCTGTCTTCGAGAATCCCAAACAAGGAAAGCTCCGCACC
381       S  T  E  S  R  H  Y  P  V  F  E  N  P  K  Q  G  K  L  R  T

                1210      1220      1230      1240      1250      1260
1201     AAGGTGGAGAACGGACAAGGCACAATCCCTGTGGAATCCAGTGACATTGTGCCTACGTGG
401       K  V  E  N  G  Q  G  T  I  P  V  E  S  S  D  I  V  P  T  W

                1270      1280      1290      1300      1310      1320
1261     GATGGCATTCGGCTCGGGGAGAGACTCCGTACCATGTCCTGTAGTGACAAAATCCTACGC
421       D  G  I  R  L  G  E  R  L  R  T  M  S  C  S  D  K  I  L  R

                1330      1340      1350      1360      1370      1380
1321     TGGAACGTGCTGGGCCTGCAAGGGGCACTGTTGACCCACTTCCTGCAGCCCATTTATCTC
441       W  N  V  L  G  L  Q  G  A  L  L  T  H  F  L  Q  P  I  Y  L

                1390      1400      1410      1420      1430      1440
1381     AAATCTGTCACATTGGGTTACCTTTTCAGCCAAGGGCATCTGACCCGTGCTATTTGCTGT
461       K  S  V  T  L  G  Y  L  F  S  Q  G  H  L  T  R  A  I  C  C

                1450      1460      1470      1480      1490      1500
1441     CGTGTGACAAGAGATGGGAGTGCATTTGAGGATGGACTACGACATCCCTTTATTGTCAAC
481       R  V  T  R  D  G  S  A  F  E  D  G  L  R  H  P  F  I  V  N

                1510      1520      1530      1540      1550      1560
1501     CACCCCAAGGTTGGCAGAGTCAGCATATATGATTCCAAAAGGCAATCCGGGAAGACTAAG
501       H  P  K  V  G  R  V  S  I  Y  D  S  K  R  Q  S  G  K  T  K

                1570      1580      1590      1600      1610      1620
1561     GAGACAAGCGTCAACTGGTGTCTGGCTGATGGCTATGACCTGGAGATCCTGGACGGTACC
521       E  T  S  V  N  W  C  L  A  D  G  Y  D  L  E  I  L  D  G  T

                1630      1640      1650      1660      1670      1680
1621     AGAGGCACTGTGGATGGGCCACGGAATGAATTGTCCCGGGTCTCCAAAAAGAACATTTTT
541       R  G  T  V  D  G  P  R  N  E  L  S  R  V  S  K  K  N  I  F

                1690      1700      1710      1720      1730      1740
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1681      CTTCTATTTAAGAAGCTCTGCTCCTTCCGTTACCGCAGGGATCTACTGAGACTCTCCTAT
561        L  L  F  K  K  L  C  S  F  R  Y  R  R  D  L  L  R  L  S  Y

                1750      1760      1770      1780      1790      1800
1741      GGTGAGGCCAAGAAAGCTGCCCGTGACTACGAGACGGCCAAGAACTACTTCAAAAAAGGC
581        G  E  A  K  K  A  A  R  D  Y  E  T  A  K  N  Y  F  K  K  G

                1810      1820      1830      1840      1850      1860
1801      CTGAAGGATATGGGCTATGGGAACTGGATTAGCAAACCCCAGGAGGAAAAGAACTTTTAT
601        L  K  D  M  G  Y  G  N  W  I  S  K  P  Q  E  E  K  N  F  Y

                1870
1861      CTCTGCCCAGTA
621        L  C  P  V
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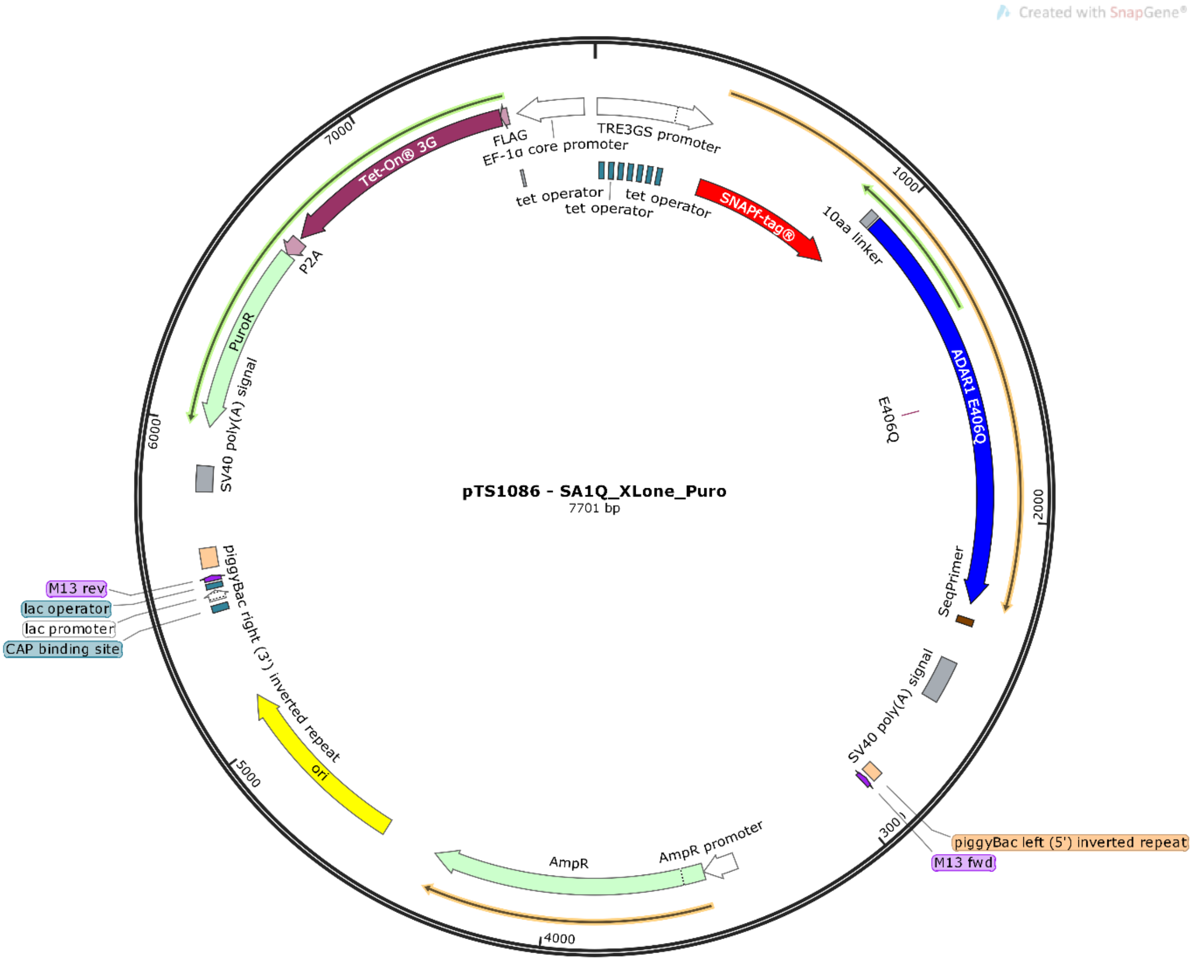
Plasmid-maps & sequences:

Plasmid-map and sequence of PiggyBac plasmid SA1Q_XLone_Puro (pTS1086): The cyan highlighted site denotes E>Q mutation in hyperactive SNAP ADAR (SA1Q). The PiggyBac plasmid SA1_XLone_Puro (pTS1074) differs from this plasmid at this site with a GAA codon (Glu) instead of CAA codon(Gln).



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121        TTACTCCCTA TCAGTGATAG AGAACGTATG ACCAGTTTAC TCCCTATCAG TGATAGAGAA
181        CGTATCTACA GTTTACTCCC TATCAGTGAT AGAGAACGTA TATCCAGTTT ACTCCCTATC
241        AGTGATAGAG AACGTATAAG CTTTGCTTAT GTAAACCAGG GCGCCTATAA AAGAGTGCTG
301        ATTTTTTGAG TAAACTTCAA TTCCACAACA CTTTTGTCTT ATACCAACTT TCCGTACCAC
361        TTCCTACCCT CGTAAAGGTA CCGCGGCCGC CACCATGGAC AAAGACTGCG AAATGAAGCG
421        CACCACCCTG GATAGCCCTC TGGGCAAGCT GGAACTGTCT GGGTGCGAAC AGGGCCTGCA
481        CCGTATCATC TTCCTGGGCA AAGGAACATC TGCCGCCGAC GCCGTGGAAG TGCCTGCCCC
541        AGCCGCCGTG CTGGGCGGAC CAGAGCCACT GATGCAGGCC ACCGCCTGGC TCAACGCCTA
601        CTTTCACCAG CCTGAGGCCA TCGAGGAGTT CCCTGTGCCA GCCCTGCACC ACCCAGTGTT
661        CCAGCAGGAG AGCTTTACCC GCCAGGTGCT GTGGAAACTG CTGAAAGTGG TGAAGTTCGG
721        AGAGGTCATC AGCTACAGCC ACCTGGCCGC CCTGGCCGGC AATCCCGCCG CCACCGCCGC
781        CGTGAAAACC GCCCTGAGCG GAAATCCCGT GCCCATTCTG ATCCCCTGCC ACCGGGTGGT
841        GCAGGGCGAC CTGGACGTGG GGGGCTACGA GGGCGGGCTC GCCGTGAAAG AGTGGCTGCT
901        GGCCCACGAG GGCCACAGAC TGGGCAAGCC TGGGCTGGGT CCTGCAGGCG GAGGCGCGCC
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961      AGGGTCTGGC GGCGGCAGTA AGGCAGAACG CATGGGTTTC ACAGAGGTAA CCCCAGTGAC
1021     AGGGGCCAGT CTCAGAAGAA CTATGCTCCT CCTCTCAAGG TCCCCAGAAG CACAGCCAAA
1081     GACACTCCCT CTCACTGGCA GCACCTTCCA TGACCAGATA GCCATGCTGA GCCACCGGTG
1141     CTTCAACACT CTGACTAACA GCTTCCAGCC CTCCTTGCTC GGCCGCAAGA TTCTGGCCGC
1201     CATCATTATG AAAAAAGACT CTGAGGACAT GGGTGTCGTC GTCAGCTTGG GAACAGGGAA
1261     TCGCTGTGTA AAAGGAGATT CTCTCAGCCT AAAAGGAGAA ACTGTCAATG ACTGCCATGC
1321     AGAAATAATC TCCCGGAGAG GCTTCATCAG GTTTCTCTAC AGTGAGTTAA TGAAATACAA
1381     CTCCCAGACT GCGAAGGATA GTATATTTGA ACCTGCTAAG GGAGGAGAAA AGCTCCAAAT
1441     AAAAAAGACT GTGTCATTCC ATCTGTATAT CAGCACTGCT CCGTGTGGAG ATGGCGCCCT
1501     CTTTGACAAG TCCTGCAGCG ACCGTGCTAT GGAAAGCACA GAATCCCGCC ACTACCCTGT
1561     CTTCGAGAAT CCCAAACAAG GAAAGCTCCG CACCAAGGTG GAGAACGGAC AAGGCACAAT
1621     CCCTGTGGAA TCCAGTGACA TTGTGCCTAC GTGGGATGGC ATTCGGCTCG GGGAGAGACT
1681     CCGTACCATG TCCTGTAGTG ACAAAATCCT ACGCTGGAAC GTGCTGGGCC TGCAAGGGGC
1741     ACTGTTGACC CACTTCCTGC AGCCCATTTA TCTCAAATCT GTCACATTGG GTTACCTTTT
1801     CAGCCAAGGG CATCTGACCC GTGCTATTTG CTGTCGTGTG ACAAGAGATG GGAGTGCATT
1861     TGAGGATGGA CTACGACATC CCTTTATTGT CAACCACCCC AAGGTTGGCA GAGTCAGCAT
1921     ATATGATTCC AAAAGGCAAT CCGGGAAGAC TAAGGAGACA AGCGTCAACT GGTGTCTGGC
1981     TGATGGCTAT GACCTGGAGA TCCTGGACGG TACCAGAGGC ACTGTGGATG GGCCACGGAA
2041     TGAATTGTCC CGGGTCTCCA AAAAGAACAT TTTTCTTCTA TTTAAGAAGC TCTGCTCCTT
2101     CCGTTACCGC AGGGATCTAC TGAGACTCTC CTATGGTGAG GCCAAGAAAG CTGCCCGTGA
2161     CTACGAGACG GCCAAGAACT ACTTCAAAAA AGGCCTGAAG GATATGGGCT ATGGGAACTG
2221     GATTAGCAAA CCCCAGGAGG AAAAGAACTT TTATCTCTGC CCAGTATAAA TCGATTCTAG
2281     TAGACCACCT CCCCTGCGAG CTAAGCTGGA CAGCCAATGA CGGGTAAGAG AGTGACATTT
2341     TTCACTAACC TAAGACAGGA GGGCCGTCAG AGCTACTGCC TAATCCAAAG ACGGGTAAAA
2401     GTGATAAAAA TGTATCACTC CAACCTAAGA CAGGCGCAGC TTCCGAGGGA TTTGAGATCC
2461     AGACATGATA AGATACATTG ATGAGTTTGG ACAAACCAAA ACTAGAATGC AGTGAAAAAA
2521     ATGCCTTATT TGTGAAATTT GTGATGCTAT TGCCTTATTT GTAACCATTA TAAGCTGCAA
2581     TAAACAAGTT TGATATCTAT AACAAGAAAA TATATATATA ATAAGTTATC ACGTAAGTAG
2641     AACATGAAAT AACAATATAA TTATCGTATG AGTTAAATCT TAAAAGTCAC GTAAAAGATA
2701     ATCATGCGTC ATTTTGACTC ACGCGGTCGT TATAGTTCAA AATCAGTGAC ACTTACCGCA
2761     TTGACAAGCA CGCCTCACGG GAGCTCCAAG CGGCGACTGA GATGTCCTAA ATGCACAGCG
2821     ACGGATTCGC GCTATTTAGA AAGAGAGAGC AATATTTCAA GAATGCATGC GTCAATTTTA
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2941     GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA CATCCCCCTT TCGCCAGCTG
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7561      CGACATCACT TTCCCAGTTT ACCCCGCGCC ACCTTCTCTA GGCACCGGTT CAATTGCCGA
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7681      CGGAGCCACT CGAGTGGAAT T
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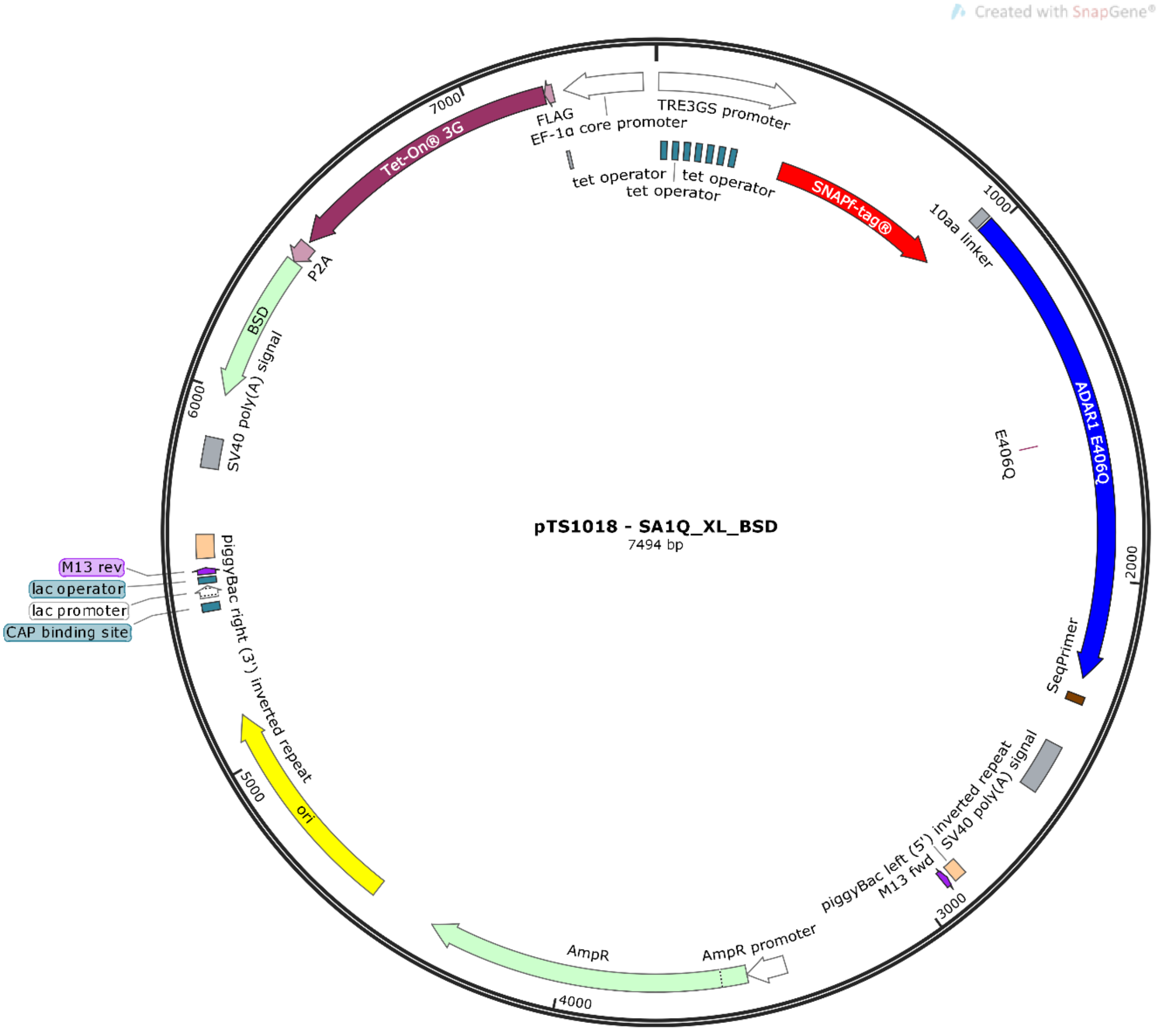
Plasmid-map and sequence of PiggyBac plasmid SA1Q_XLone_BSD (pTS1018): The cyan highlighted site denotes E>Q mutation in hyperactive SNAP ADAR (SA1Q). The PiggyBac plasmid SA1_XLone_BSD (pTS990) differs from this plasmid at this site with a GAA codon (Glu) instead of CAA codon (Gln).



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901       GGCCCACGAG GGCCACAGAC TGGGCAAGCC TGGGCTGGGT CCTGCAGGCG GAGGCGCGCC
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1081     GACACTCCCT CTCACTGGCA GCACCTTCCA TGACCAGATA GCCATGCTGA GCCACCGGTG
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1981     TGATGGCTAT GACCTGGAGA TCCTGGACGG TACCAGAGGC ACTGTGGATG GGCCACGGAA
2041     TGAATTGTCC CGGGTCTCCA AAAAGAACAT TTTTCTTCTA TTTAAGAAGC TCTGCTCCTT
2101     CCGTTACCGC AGGGATCTAC TGAGACTCTC CTATGGTGAG GCCAAGAAAG CTGCCCGTGA
2161     CTACGAGACG GCCAAGAACT ACTTCAAAAA AGGCCTGAAG GATATGGGCT ATGGGAACTG
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2281     TAGACCACCT CCCCTGCGAG CTAAGCTGGA CAGCCAATGA CGGGTAAGAG AGTGACATTT
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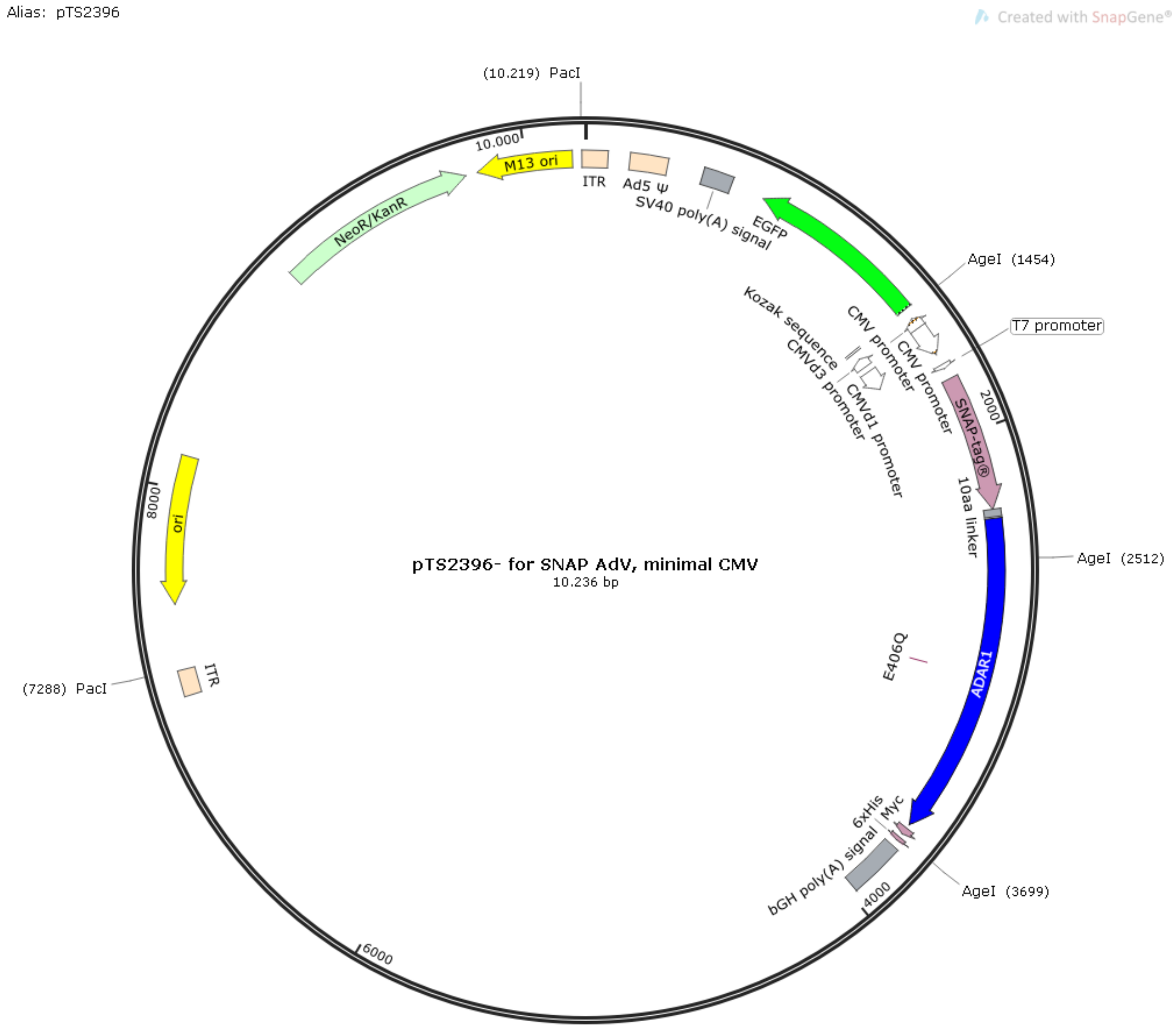

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1381    GTCGCCGTCC AGCTCGACCA GGATGGGCAC CACCCCGGTG AACAGCTCCT CGCCCTTGCT
1441    CACCATGGTG GCGACCGGTA GCGCTAGCGG ATCTGACGGT TCACTAAACC AGCTCTGCTT
1501    ATATAGACCT CCCACCGTAC ACGCCTACCG CCCATTGACG TCAATGGGAG TTTGTTTTGG
1561    CACCAAAATC AACGGGACTT TCCAAAATGT CGTAACAACT CCGCCCCATT GACGCAAATG
1621    GGCGGTAGGC GTGTACGGTG GGAGGTCTAT ATAAGCAGAG CTCTCTGGCT AACTAGAGAA
1681    CCCACTGCTT ACTGGCTTAT CGAAATTAAT ACGACTCACT ATAGGGAGAC CCAAGCTGGC
1741    TAGTTAAGCT TGGTACCGAG CTCGGATCCA CCATGGACAA AGACTGCGAA ATGAAGCGCA
1801    CCACCCTGGA TAGCCCTCTG GGCAAGCTGG AACTGTCTGG GTGCGAACAG GGCCTGCACC
1861    GTATCATCTT CCTGGGCAAA GGAACATCTG CCGCCGACGC CGTGGAAGTG CCTGCCCCAG
1921    CCGCCGTGCT GGGCGGACCA GAGCCACTGA TGCAGGCCAC CGCCTGGCTC AACGCCTACT
1981    TTCACCAGCC TGAGGCCATC GAGGAGTTCC CTGTGCCAGC CCTGCACCAC CCAGTGTTCC
2041    AGCAGGAGAG CTTTACCCGC CAGGTGCTGT GGAAACTGCT GAAAGTGGTG AAGTTCGGAG
2101    AGGTCATCAG CTACAGCCAC CTGGCCGCCC TGGCCGGCAA TCCCGCCGCC ACCGCCGCCG
2161    TGAAAACCGC CCTGAGCGGA AATCCCGTGC CCATTCTGAT CCCCTGCCAC CGGGTGGTGC
2221    AGGGCGACCT GGACGTGGGG GGCTACGAGG GCGGGCTCGC CGTGAAAGAG TGGCTGCTGG
2281    CCCACGAGGG CCACAGACTG GGCAAGCCTG GGCTGGGTCC TGCAGGCGGA GGCGCGCCAG
2341    GGTCTGGCGG CGGCAGTAAG GCAGAACGCA TGGGTTTCAC AGAGGTAACC CCAGTGACAG
2401    GGGCCAGTCT CAGAAGAACT ATGCTCCTCC TCTCAAGGTC CCCAGAAGCA CAGCCAAAGA
2461    CACTCCCTCT CACTGGCAGC ACCTTCCATG ACCAGATAGC CATGCTGAGC CACCGGTGCT
2521    TCAACACTCT GACTAACAGC TTCCAGCCCT CCTTGCTCGG CCGCAAGATT CTGGCCGCCA
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2641    GCTGTGTAAA AGGAGATTCT CTCAGCCTAA AAGGAGAAAC TGTCAATGAC TGCCATGCAG
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3421    AATTGTCCCG GGTCTCCAAA AAGAACATTT TTCTTCTATT TAAGAAGCTC TGCTCCTTCC
3481    GTTACCGCAG GGATCTACTG AGACTCTCCT ATGGTGAGGC CAAGAAAGCT GCCCGTGACT
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3721    ATTGAGTTGA AACCCGCTGA TCAGCCTCGA CTGTGCCTTC TAGTTGCCAG CCATCTGTTG
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4201    CTGCCCGCAA ACTCTACTAC CTTGACCTAC GAGACCGTGT CTGGAACGCC GTTGGAGACT
4261    GCAGCCTCCG CCGCCGCTTC AGCCGCTGCA GCCACCGCCC GCGGGATTGT GACTGACTTT
4321    GCTTTCCTGA GCCCGCTTGC AAGCAGTGCA GCTTCCCGTT CATCCGCCCG CGATGACAAG
4381    TTGACGGCTC TTTTGGCACA ATTGGATTCT TTGACCCGGG AACTTAATGT CGTTTCTCAG
4441    CAGCTGTTGG ATCTGCGCCA GCAGGTTTCT GCCCTGAAGG CTTCCTCCCC TCCCAATGCG
4501    GTTTAAAACA TAAATAAAAA ACCAGACTCT GTTTGGATTT GGATCAAGCA AGTGTCTTGC
4561    TGTCTTTATT TAGGGGTTTT GCGCGCGCGG TAGGCCCGGG ACCAGCGGTC TCGGTCGTTG
4621    AGGGTCCTGT GTATTTTTTC CAGGACGTGG TAAAGGTGAC TCTGGATGTT CAGATACATG
4681    GGCATAAGCC CGTCTCTGGG GTGGAGGTAG CACCACTGCA GAGCTTCATG CTGCGGGGTG
4741    GTGTTGTAGA TGATCCAGTC GTAGCAGGAG CGCTGGGCGT GGTGCCTAAA AATGTCTTTC
4801    AGTAGCAAGC TGATTGCCAG GGGCAGGCCC TTGGTGTAAG TGTTTACAAA GCGGTTAAGC
4861    TGGGATGGGT GCATACGTGG GGATATGAGA TGCATCTTGG ACTGTATTTT TAGGTTGGCT
4921    ATGTTCCCAG CCATATCCCT CCGGGGATTC ATGTTGTGCA GAACCACCAG CACAGTGTAT
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5101     CGGGCGGCGG CCTGGGCGAA GATATTTCTG GGATCACTAA CGTCATAGTT GTGTTCCAGG
5161     ATGAGATCGT CATAGGCCAT TTTTACAAAG CGCGGGCGGA GGGTGCCAGA CTGCGGTATA
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5461     TCATCCCTGA GCAGGGGGGC CACTTCGTTA AGCATGTCCC TGACTCGCAT GTTTTCCCTG
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5581     TTTTTCAACG GTTTGAGACC GTCCGCCGTA GGCATGCTTT TGAGCGTTTG ACCAAGCAGT
5641     TCCAGGCGGT CCCACAGCTC GGTCACCTGC TCTACGGCAT CTCGATCCAG CATATCTCCT
5701     CGTTTCGCGG GTTGGGGCGG CTTTCGCTGT ACGGCAGTAG TCGGTGCTCG TCCAGACGGG
5761     CCAGGGTCAT GTCTTTCCAC GGGCGCAGGG TCCTCGTCAG CGTAGTCTGG GTCACGGTGA
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5881     TGAAGCGCTG CCGGTCTTCG CCCTGCGCGT CGGCCAGGTA GCATTTGACC ATGGTGTCAT
5941     AGTCCAGCCC CTCCGCGGCG TGGCCCTTGG CGCGCAGCTT GCCCTTGGAG GAGGCGCCGC
6001     ACGAGGGGCA GTGCAGACTT TTGAGGGCGT AGAGCTTGGG CGCGAGAAAT ACCGATTCCG
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6121     GCTCTGGCCG TTCGGGGTCA AAAACCAGGT TTCCCCCATG CTTTTTGATG CGTTTCTTAC
6181     CTCTGGTTTC CATGAGCCGG TGTCCACGCT CGGTGACGAA AAGGCTGTCC GTGTCCCCGT
6241     ATACAGACTT GAGAGGGAGT TTAAACGAAT TCAATAGCTT GTTGCATGGG CGGCGATATA
6301     AAATGCAAGG TGCTGCTCAA AAAATCAGGC AAAGCCTCGC GCAAAAAAGA AAGCACATCG
6361     TAGTCATGCT CATGCAGATA AAGGCAGGTA AGCTCCGGAA CCACCACAGA AAAAGACACC
6421     ATTTTTCTCT CAAACATGTC TGCGGGTTTC TGCATAAACA CAAAATAAAA TAACAAAAAA
6481     ACATTTAAAC ATTAGAAGCC TGTCTTACAA CAGGAAAAAC AACCCTTATA AGCATAAGAC
6541     GGACTACGGC CATGCCGGCG TGACCGTAAA AAAACTGGTC ACCGTGATTA AAAAGCACCA
6601     CCGACAGCTC CTCGGTCATG TCCGGAGTCA TAATGTAAGA CTCGGTAAAC ACATCAGGTT
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6781     ACACATAAAC ACCTGAAAAA CCCTCCTGCC TAGGCAAAAT AGCACCCTCC CGCTCCAGAA
6841     CAACATACAG CGCTTCCACA GCGGCAGCCA TAACAGTCAG CCTTACCAGT AAAAAAGAAA
6901     ACCTATTAAA AAAACACCAC TCGACACGGC ACCAGCTCAA TCAGTCACAG TGTAAAAAAG
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7141     CTACAATTCC CAACACATAC AAGTTACTCC GCCCTAAAAC CTACGTCACC CGCCCCGTTC
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7381     TGACTCGCTG CGCTCGGTCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGCGGT
7441     AATACGGTTA TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA
7501     GCAAAAGGCC AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC
7561     CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT
7621     ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT
7681     GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCATAG
7741     CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA
7801     CGAACCCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA
7861     CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC
7921     GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG
7981     AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG
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8281     TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT
8341     CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG
8401     GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC GCTCACCGGC
8461     TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC
8521     AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC
8581     GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTGCA GCCATGAGAT TATCAAAAAG
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8641     GATCTTCACC TAGATCCTTT TCACGTAGAA AGCCAGTCCG CAGAAACGGT GCTGACCCCG
8701     GATGAATGTC AGCTACTGGG CTATCTGGAC AAGGGAAAAC GCAAGCGCAA AGAGAAAGCA
8761     GGTAGCTTGC AGTGGGCTTA CATGGCGATA GCTAGACTGG GCGGTTTTAT GGACAGCAAG
8821     CGAACCGGAA TTGCCAGCTG GGGCGCCCTC TGGTAAGGTT GGGAAGCCCT GCAAAGTAAA
8881     CTGGATGGCT TTCTTGCCGC CAAGGATCTG ATGGCGCAGG GGATCAAGCT CTGATCAAGA
8941     GACAGGATGA GGATCGTTTC GCATGATTGA ACAAGATGGA TTGCACGCAG GTTCTCCGGC
9001     CGCTTGGGTG GAGAGGCTAT TCGGCTATGA CTGGGCACAA CAGACAATCG GCTGCTCTGA
9061     TGCCGCCGTG TTCCGGCTGT CAGCGCAGGG GCGCCCGGTT CTTTTTGTCA AGACCGACCT
9121     GTCCGGTGCC CTGAATGAAC TGCAAGACGA GGCAGCGCGG CTATCGTGGC TGGCCACGAC
9181     GGGCGTTCCT TGCGCAGCTG TGCTCGACGT TGTCACTGAA GCGGGAAGGG ACTGGCTGCT
9241     ATTGGGCGAA GTGCCGGGGC AGGATCTCCT GTCATCTCAC CTTGCTCCTG CCGAGAAAGT
9301     ATCCATCATG GCTGATGCAA TGCGGCGGCT GCATACGCTT GATCCGGCTA CCTGCCCATT
9361     CGACCACCAA GCGAAACATC GCATCGAGCG AGCACGTACT CGGATGGAAG CCGGTCTTGT
9421     CGATCAGGAT GATCTGGACG AAGAGCATCA GGGGCTCGCG CCAGCCGAAC TGTTCGCCAG
9481     GCTCAAGGCG AGCATGCCCG ACGGCGAGGA TCTCGTCGTG ACCCATGGCG ATGCCTGCTT
9541     GCCGAATATC ATGGTGGAAA ATGGCCGCTT TTCTGGATTC ATCGACTGTG GCCGGCTGGG
9601     TGTGGCGGAC CGCTATCAGG ACATAGCGTT GGCTACCCGT GATATTGCTG AAGAGCTTGG
9661     CGGCGAATGG GCTGACCGCT TCCTCGTGCT TTACGGTATC GCCGCTCCCG ATTCGCAGCG
9721     CATCGCCTTC TATCGCCTTC TTGACGAGTT CTTCTGAATT TTGTTAAAAT TTTTGTTAAA
9781     TCAGCTCATT TTTTAACCAA TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAAGAAT
9841     AGACCGAGAT AGGGTTGAGT GTTGTTCCAG TTTGGAACAA GAGTCCACTA TTAAAGAACG
9901     TGGACTCCAA CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA CTACGTGAAC
9961     CATCACCCTA ATCAAGTTTT TTGGGGTCGA GGTGCCGTAA AGCACTAAAT CGGAACCCTA
10021    AAGGGAGCCC CCGATTTAGA GCTTGACGGG GAAAGCCGGC GAACGTGGCG AGAAAGGAAG
10081    GGAAGAAAGC GAAAGGAGCG GGCGCTAGGG CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG
10141    TAACCACCAC ACCCGCCGCG CTTAATGCGC CGCTACAGGG CGCGTCCATT CGCCATTCAG
10201    GATCGAATTA ATTCTTAATT AACATCATCA ATAATA
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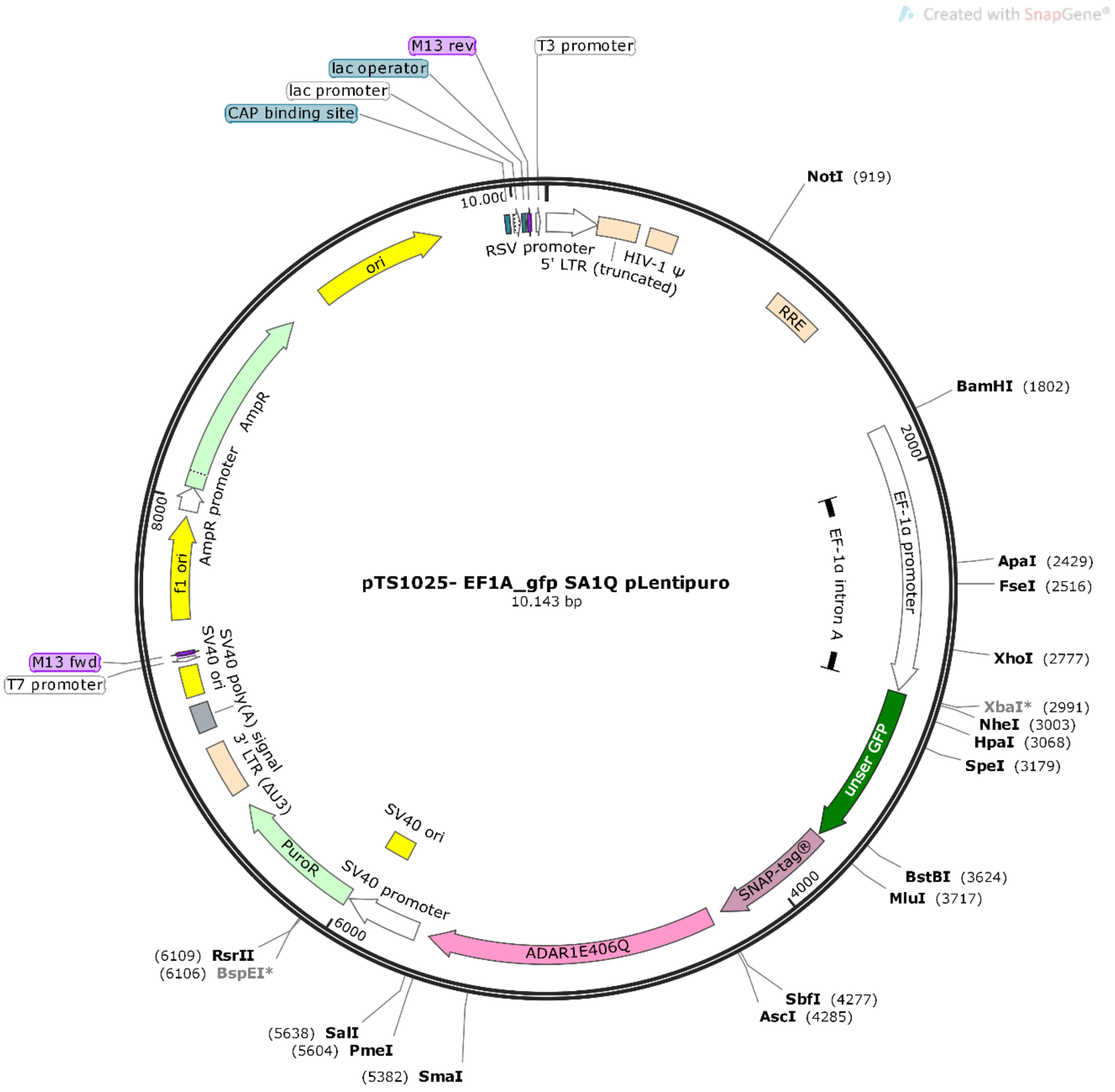
Plasmid-map and sequence of EF1A_GFP_SA1Q_pLentiPuro (pTS1025). E>Q site is highlighted in cyan:



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1          ATGTAGTCTT ATGCAATACT CTTGTAGTCT TGCAACATGG TAACGATGAG TTAGCAACAT
61         GCCTTACAAG GAGAGAAAAA GCACCGTGCA TGCCGATTGG TGGAAGTAAG GTGGTACGAT
121        CGTGCCTTAT TAGGAAGGCA ACAGACGGGT CTGACATGGA TTGGACGAAC CACTGAATTG
181        CCGCATTGCA GAGATATTGT ATTTAAGTGC CTAGCTCGAT ACATAAACGG GTCTCTCTGG
241        TTAGACCAGA TCTGAGCCTG GGAGCTCTCT GGCTAACTAG GGAACCCACT GCTTAAGCCT
301        CAATAAAGCT TGCCTTGAGT GCTTCAAGTA GTGTGTGCCC GTCTGTTGTG TGACTCTGGT
361        AACTAGAGAT CCCTCAGACC CTTTTAGTCA GTGTGGAAAA TCTCTAGCAG TGGCGCCCGA
421        ACAGGGACTT GAAAGCGAAA GGGAAACCAG AGGAGCTCTC TCGACGCAGG ACTCGGCTTG
481        CTGAAGCGCG CACGGCAAGA GGCGAGGGGC GGCGACTGGT GAGTACGCCA AAAATTTTGA
541        CTAGCGGAGG CTAGAAGGAG AGAGATGGGT GCGAGAGCGT CAGTATTAAG CGGGGGAGAA
601        TTAGATCGCG ATGGGAAAAA ATTCGGTTAA GGCCAGGGGG AAAGAAAAAA TATAAATTAA
661        AACATATAGT ATGGGCAAGC AGGGAGCTAG AACGATTCGC AGTTAATCCT GGCCTGTTAG
721        AAACATCAGA AGGCTGTAGA CAAATACTGG GACAGCTACA ACCATCCCTT CAGACAGGAT
781        CAGAAGAACT TAGATCATTA TATAATACAG TAGCAACCCT CTATTGTGTG CATCAAAGGA
841        TAGAGATAAA AGACACCAAG GAAGCTTTAG ACAAGATAGA GGAAGAGCAA AACAAAAGTA
901        AGACCACCGC ACAGCAAGCG GCCGCTGATC TTCAGACCTG GAGGAGGAGA TATGAGGGAC
961        AATTGGAGAA GTGAATTATA TAAATATAAA GTAGTAAAAA TTGAACCATT AGGAGTAGCA
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1141      ACGGTACAGG CCAGACAATT ATTGTCTGGT ATAGTGCAGC AGCAGAACAA TTTGCTGAGG
1201      GCTATTGAGG CGCAACAGCA TCTGTTGCAA CTCACAGTCT GGGGCATCAA GCAGCTCCAG
1261      GCAAGAATCC TGGCTGTGGA AAGATACCTA AAGGATCAAC AGCTCCTGGG GATTTGGGGT
1321      TGCTCTGGAA AACTCATTTG CACCACTGCT GTGCCTTGGA ATGCTAGTTG GAGTAATAAA
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1741      GGAGAGAGAG ACAGAGACAG ATCCATTCGA TTAGTGAACG GATCTCGACG GTATCGCTAG
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1861      AGAAGTTGGG GGGAGGGGTC GGCAATTGAA CCGGTGCCTA GAGAAGGTGG CGCGGGGTAA
1921      ACTGGGAAAG TGATGTCGTG TACTGGCTCC GCCTTTTTCC CGAGGGTGGG GGAGAACCGT
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2161      GAAGTGGGTG GGAGAGTTCG AGGCCTTGCG CTTAAGGAGC CCCTTCGCCT CGTGCTTGAG
2221      TTGAGGCCTG GCCTGGGCGC TGGGGCCGCC GCGTGCGAAT CTGGTGGCAC CTTCGCGCCT
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2341      TTTTTTTCTG GCAAGATAGT CTTGTAAATG CGGGCCAAGA TCTGCACACT GGTATTTCGG
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2461      GGGGCCTGCG AGCGCGGCCA CCGAGAATCG GACGGGGGTA GTCTCAAGCT GGCCGGCCTG
2521      CTCTGGTGCC TGGCCTCGCG CCGCCGTGTA TCGCCCCGCC CTGGGCGGCA AGGCTGGCCC
2581      GGTCGGCACC AGTTGCGTGA GCGGAAAGAT GGCCGCTTCC CGGCCCTGCT GCAGGGAGCT
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2761      GGCACCTCGA TTAGTTCTCG AGCTTTTGGA GTACGTCGTC TTTAGGTTGG GGGGAGGGGT
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3241      GGCATGACTT TTTCAAGAGT GCCATGCCCG AAGGTTATGT ACAGGAAAGG ACCATCTTCT
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3781      TGGAACTGTC TGGGTGCGAA CAGGGCCTGC ACCGTATCAT CTTCCTGGGC AAAGGAACAT
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3901      TGATGCAGGC CACCGCCTGG CTCAACGCCT ACTTTCACCA GCCTGAGGCC ATCGAGGAGT
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4021      TGTGGAAACT GCTGAAAGTG GTGAAGTTCG GAGAGGTCAT CAGCTACAGC CACCTGGCCG
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4621      TAAAAGGAGA AACTGTCAAT GACTGCCATG CAGAAATAAT CTCCCGGAGA GGCTTCATCA
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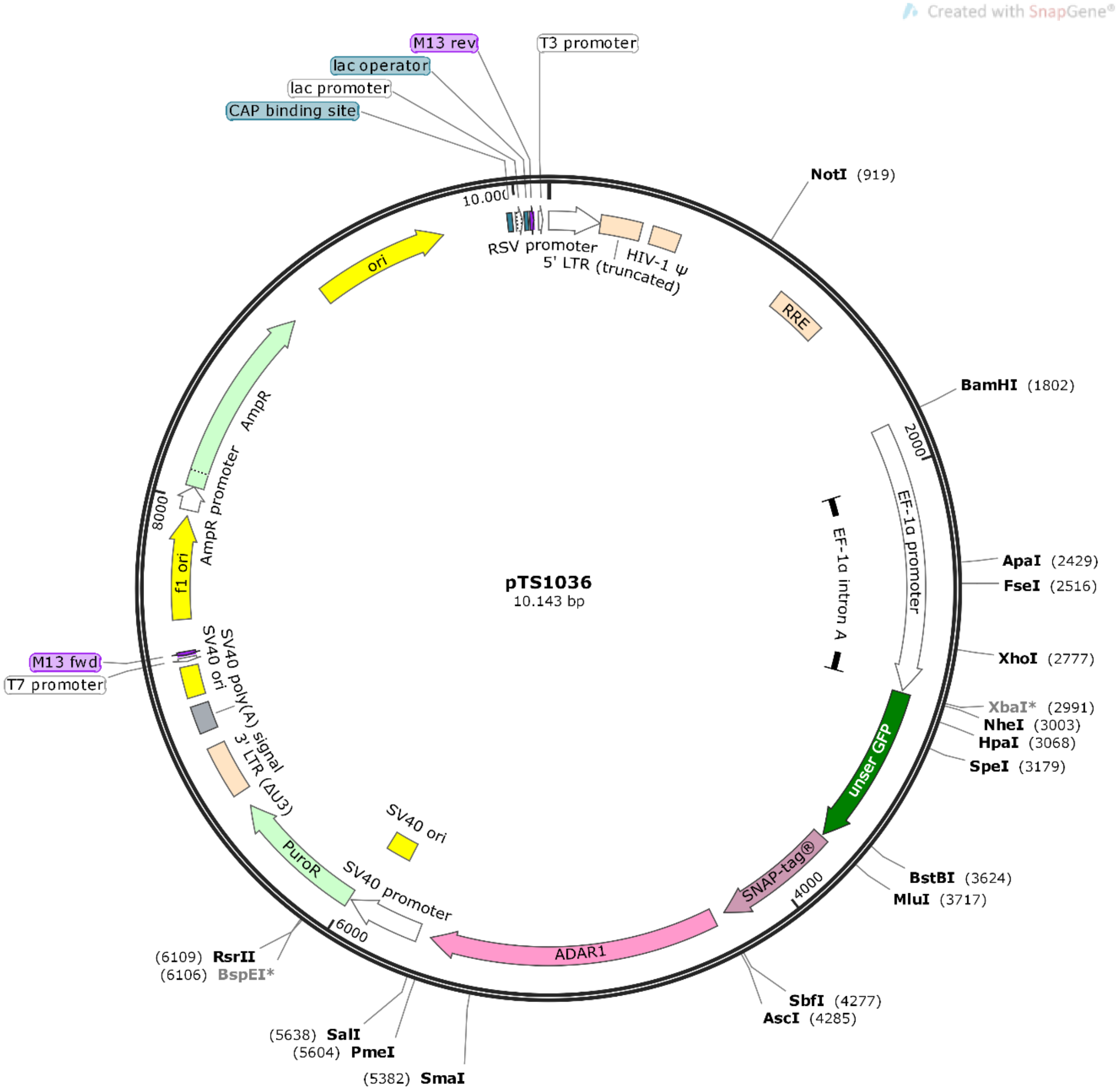

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8641     AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG GATAAAGTTG
8701     CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG
8761     CCGGTGAGCG TGGGTCTCGC GGTATCATTG CAGCACTGGG GCCAGATGGT AAGCCCTCCC
8821     GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA
8881     TCGCTGAGAT AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT
8941     ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG GTGAAGATCC
9001     TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG
9061     ACCCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC GTAATCTGCT
9121     GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC
9181     CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTTCTTC
9241     TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT ACATACCTCG
9301     CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT
9361     TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG GGGGGTTCGT
9421     GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC
9481     TATGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA
9541     GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG TATCTTTATA
9601     GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG
9661     GGCGGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT
9721     GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA
9781     CCGCCTTTGA GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG
9841     TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG CGTTGGCCGA
9901     TTCATTAATG CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG
9961     CAATTAATGT GAGTTAGCTC ACTCATTAGG CACCCCAGGC TTTACACTTT ATGCTTCCGG
10021    CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC
10081    ATGATTACGC CAAGCGCGCA ATTAACCCTC ACTAAAGGGA ACAAAAGCTG GAGCTGCAAG
10141    CTT
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