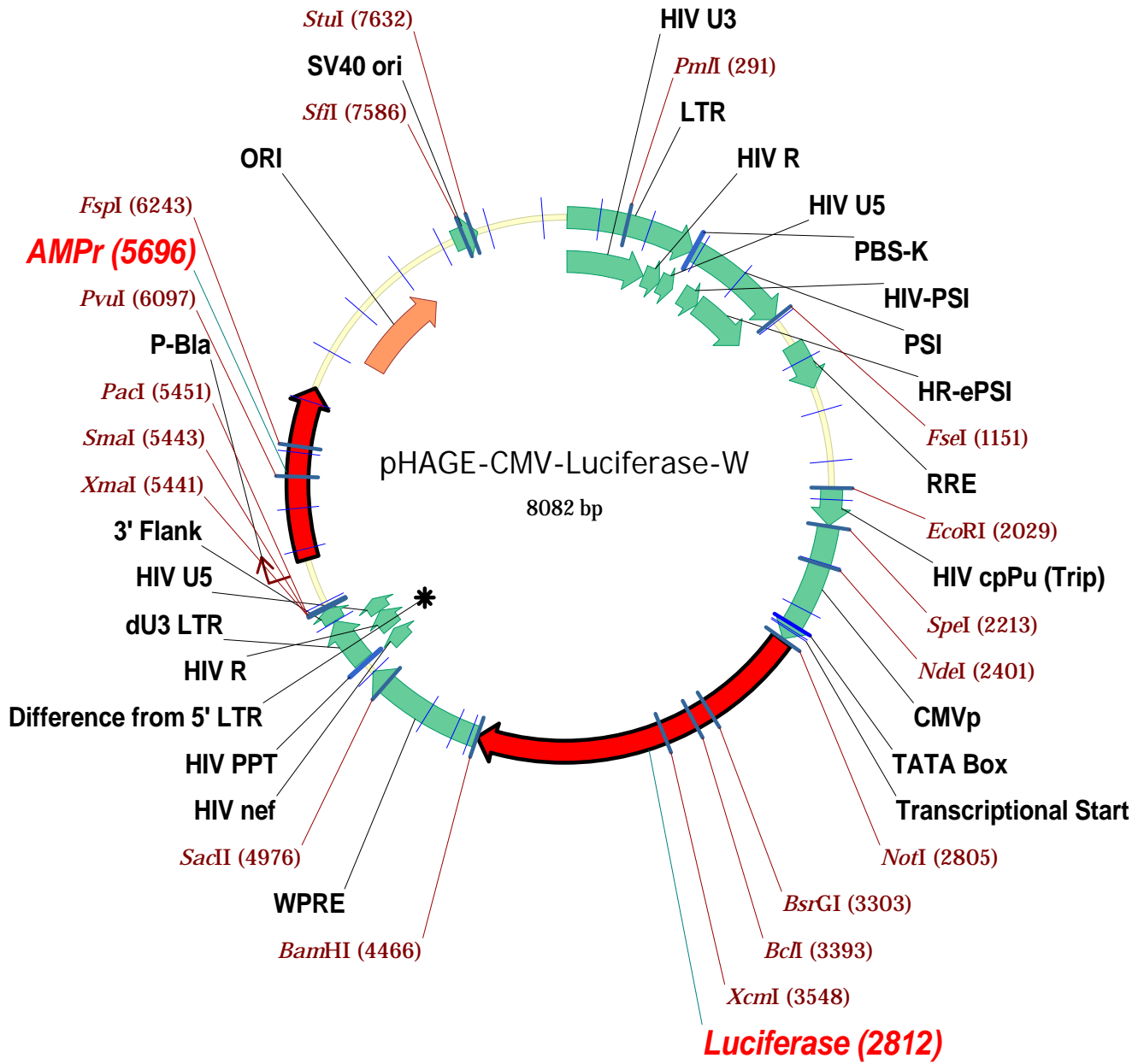


pHAGE-CMV-Luciferase-W



General Description

DNA pHAGE-CMV-Luciferase-W
Entire molecule length: 8082 bp

Standard Fields

Original Source Database: GenBank
Division: SYN
Modification Date in the Original DB: 31-JUL-2003
Original Accession Number(s): Your; mama'
Sequence Source: Unknown.
Organism(s): Unknown(Unknown)
Taxonomy: Unclassified
Associated Genes: LTR; psi; rre; cppt; CMVp; eGFP; WPRE; dU3 LTR; 3'flank; AMPr; ORI;

User Fields

Author(s)

Author
Alex Balazs
Original author

References

REFERENCE 1 (bases 1 to 8541)
AUTHORS Self
JOURNAL Unpublished.

Comments

HRST Ligated into PUC
WPRE fixed
Changed cPPT to include SpeI site for cloning promoters
cloned in clean CMV promoter without extraneous sequence
9/19/03 Completely sequenced with 4x coverage to confirm perfect sequence
11/13/03 Confirmed shortened cloning to add SmaI/PacI and tag
11/20/03 Tested Virus production and expression from viral integration in parallel with all Intermediate
12/29/03 Sequencing of Luciferase insert finds missing A at 4438 leaving no stop codon

Feature Map

CDS (2 total)

Luciferase

Start: 2812 End: 4464

AMPr

Start: 5696 End: 6535

Ampicillin Resistance Gene

Original Location Description:

6153..6992

Qualifiers:

/gene="AMPr"

/product="beta-lactamase (mature form)"

Misc. Feature (19 total)

LTR

Start: 1 End: 636

Long Terminal Repeat

Original Location Description:

1..636

Qualifiers:

/gene="LTR"

HIV U3

Start: 1 End: 453

Full Length HIV U3

HIV R

Start: 454 End: 551

HIV Repeat Region

HIV U5

Start: 552 End: 636

HIV U5

PBS-K

Start: 636 End: 658

tRNA binding site for Lysine tRNA

PSI

Start: 637 End: 1155

PSI Packaging Sequence

Original Location Description:

637..1155

Qualifiers:

/gene="psi"

/product="pbs-gag"

HIV-PSI

Start: 697 End: 806

HR-ePSI

Start: 807 End: 1144

RRE

Start: 1303 End: 1536

Rev Responsive Element

Original Location Description:

1303..1536

Qualifiers:

/gene="rre"

/product="minimal RRE"

HIV cpPu (Trip)

Start: 2034 End: 2211

Central Poly Purine Track

Original Location Description:

2034..2211

Qualifiers:

/gene="cppt"

CMVp

Start: 2218 End: 2802

Cytomegalovirus Promoter

Original Location Description:

2225..2741

Qualifiers:

/gene="CMVp"

/product="CMV IE promoter"

WPRE

Start: 4471 End: 5062

Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element

Original Location Description:

3563..4154
Qualifiers:
/gene="WPRE"

HIV nef

Start: 5068 End: 5156
HIV nef

HIV PPT

Start: 5118 End: 5132
Polypurene Tract (Not degraded by RNAseH so it can serve as a primer for + strand DNA

dU3 LTR

Start: 5133 End: 5368
Deleted U3 Long-Terminal Repeat
Original Location Description:
4225..4460
Qualifiers:
/gene="dU3 LTR"

HIV R

Start: 5170 End: 5285
HIV Repeat Region

HIV U5

Start: 5286 End: 5368
HIV U5

3' Flank

Start: 5369 End: 5439
Chromosomal 3' Flanking Sequence carried over from original HIV integration site

SV40 ori

Start: 7534 End: 7657
Original Location Description:
7991..8114
Qualifiers:
/gene="SV40 ori"

Modified Base (1 total)

Difference from 5' LTR

Start: 5181 End: 5181
This T is a C in the 5' LTR

Primer Binding Site (28 total)

pHAGE-A0050-8050

Start: 158 End: 179 (Complementary)

pHAGE-S501-1000

Start: 401 End: 421

pHAGE-A0550-0050

Start: 667 End: 690 (Complementary)

pHAGE-S1001-1500

Start: 898 End: 919

pHAGE-A1050-0550

Start: 1166 End: 1189 (Complementary)

pHAGE-S1501-2000

Start: 1391 End: 1417

pHAGE-A1550-1050

Start: 1650 End: 1678 (Complementary)
pHAGE-S2001-2500
Start: 1898 End: 1922
pHAGE-A2050-1550
Start: 2084 End: 2112 (Complementary)
pHAGE-S2501-3000
Start: 2393 End: 2417
pHAGE-A2550-2050
Start: 2632 End: 2655 (Complementary)
pHAGE 5' CDS
Start: 2746 End: 2766
pHAGE 3' CDS
Start: 4499 End: 4533 (Complementary)
pHAGE-A3550-3050
Start: 4577 End: 4600 (Complementary)
pHAGE-S4001-4500
Start: 4730 End: 4750
pHAGE-A4050-3550
Start: 5058 End: 5080 (Complementary)
pHAGE-S4501-5000
Start: 5366 End: 5397
pHAGE-S6001-6500
Start: 5472 End: 5494
pHAGE-A6050-5550
Start: 5745 End: 5768 (Complementary)
pHAGE-S6501-7000
Start: 5945 End: 5969
pHAGE-A6550-6050
Start: 6214 End: 6237 (Complementary)
pHAGE-S7001-7500
Start: 6465 End: 6489
pHAGE-A7050-6550
Start: 6725 End: 6746 (Complementary)
pHAGE-S7501-8000
Start: 6969 End: 6993
pHAGE-A7550-7050
Start: 7236 End: 7257 (Complementary)
pHAGE-S8001-8500
Start: 7472 End: 7491
pHAGE-A8050-7550
Start: 7712 End: 7738 (Complementary)
pHAGE-S1-500
Start: 7977 End: 8005

Promoter Prokaryotic (1 total)

P-Bla

Start: 5643 End: 5677
Beta Lactamase Promoter

Replication Origin (1 total)

ORI

Start: 6764 End: 7297
Bacterial Origin of Replication
Original Location Description:
7221..7754
Qualifiers:
/gene="ORI"
/product="ColE1 origin of replication"

TATA Signal (1 total)

TATA Box

Start: 2722 End: 2726
CMV Promoter TATA Box

Primary Transcript (1 total)

Transcriptional Start

Start: 2754 End: 2754
CMV Transcriptional Start Site

Restriction/Methylation Map

Enzyme	# of cuts	Positions
ApaLI	3	4682 5794 7040
AvaI	4	296 1928 3869 5441
BamHI	1	4466
EcoRI	1	2029
HindIII	4	532 1088 1676 5266
NcoI	2	2527 2811
SmaI	1	5443
XmaI	1	5441

No cuts: ClaI, PstI

Sequence

```
1  tggaaagggt aattcactcc caaagaagac aagatatacct tgatctgtgg atctaccaca
cacaaggcta cttccctgat tagcagaact acacaccagg
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agataaggta gaagaggcca ataaaggaga gaacaccagc
201 ttgttacacc ctgtgagcct gcatgggatg gatgaccgg agagagaagt gttagagtgg
aggtttgaca gccgcctagc attcatcac gtggcccag
301 agctgcatcc ggagtacttc aagaactgct gatatcgagc ttgctacaag ggactttccg
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Component Fragments