



General Description

DNA pHAGE-CMV-DsRed Express-UBC-eGFP-W
Entire molecule length: 8246 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

Original author

A Omari

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JOURNAL Unpublished.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
10/08/03 Mutations found in IRES Element Between BamHI and NdeI sites
11/03/03 After Re-cloning the IRES from TOPO vector sequence is now confirmed to be perfect (IRES contains 7As at one point which seems to only be 6As in MCV genome)
11/13/03 Confirmed shortened cloning to add SmaI/PacI and tag
11/24/03 Sequencing confirms perfect DsRed Express insert sequence
03/16/04 DK UBC cloned into 2nd promoter position, sequencing confirmed, then CMV promoter
Sequencing confirms perfect CMVp insert from cpPU through UBC 2nd promoter position.

Feature Map

CDS (3 total)

DsRed Express

Start: 2812 End: 3489

DsRed Express Red Fluorescent Protein

eGFP

Start: 3903 End: 4622

AMPr

Start: 5860 End: 6699

Ampicillin Resistance Gene

Original Location Description:
6153..6992
Qualifiers:
/gene="AMPr"
/product="beta-lactamase (mature form)"

Misc. Feature (18 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: 4635 End: 5226
Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element
Original Location Description:
3563..4154
Qualifiers:
/gene="WPRE"

HIV nef

Start: 5232 End: 5320
HIV nef

dU3 LTR

Start: 5297 End: 5532
Deleted U3 Long-Terminal Repeat
Original Location Description:
4225..4460
Qualifiers:
/gene="dU3 LTR"

HIV R

Start: 5334 End: 5449
HIV Repeat Region

HIV U5

Start: 5450 End: 5532
HIV U5

3' Flank

Start: 5533 End: 5603
Chromosomal 3' Flanking Sequence carried over from original HIV integration site

SV40 ori

Start: 7698 End: 7821
Original Location Description:
7991..8114
Qualifiers:
/gene="SV40 ori"

Modified Base (1 total)

Difference from 5' LTR
Start: 5345 End: 5345
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: 4663 End: 4697 (Complementary)
pHAGE-A3550-3050
Start: 4741 End: 4764 (Complementary)
pHAGE-S4001-4500
Start: 4894 End: 4914
pHAGE-A4050-3550
Start: 5222 End: 5244 (Complementary)
pHAGE-S4501-5000
Start: 5530 End: 5561
pHAGE-S6001-6500
Start: 5636 End: 5658
pHAGE-A6050-5550
Start: 5909 End: 5932 (Complementary)
pHAGE-S6501-7000
Start: 6109 End: 6133
pHAGE-A6550-6050
Start: 6378 End: 6401 (Complementary)
pHAGE-S7001-7500
Start: 6629 End: 6653
pHAGE-A7050-6550
Start: 6889 End: 6910 (Complementary)
pHAGE-S7501-8000
Start: 7133 End: 7157
pHAGE-A7550-7050
Start: 7400 End: 7421 (Complementary)
pHAGE-S8001-8500
Start: 7636 End: 7655

pHAGE-A8050-7550

Start: 7876 End: 7902 (Complementary)

pHAGE-S1-500

Start: 8141 End: 8169

Promoter Eukaryotic (1 total)

UBCp

Start: 3491 End: 3901

Promoter Prokaryotic (1 total)

P-Bla

Start: 5807 End: 5841
Beta Lactamase Promoter

Replication Origin (1 total)

ORI

Start: 6928 End: 7461
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
AfIII	2	518 5416
ApaLI	3	4846 5958 7204
AvaI	4	296 1928 3074 5605
BamHI	1	3491
BglII	4	474 5265 5331 5372
BsrGI	1	4613
Clal	1	4625
DraI	6	2062 5282 6052 6744 6763 8204
EagI	3	1145 1149 2805
EcoRI	1	2029
EcoRV	3	36 115 334
EgeI	3	640 3518 3531
FseI	1	1151

Fspl	2	2861 6407
HindIII	4	532 1088 1676 5430
MscI	1	3359
NaeI	2	1149 5151
NcoI	2	2527 2811
NdeI	2	2401 3902
NotI	1	2805
PacI	1	5615
PmlI	1	291
PstI	1	3153
PvuI	1	6261
PvuII	3	436 3376 5260
SacI	4	492 683 2735 5390
SacII	2	3527 5140
SbfI	1	3153
SdaI	1	3153
SfiI	2	2813 7750
SmaI	1	5607
SpeI	1	2213
StuI	2	3245 7796
XmaI	1	5605

No cuts: Acc65I, AscI, BclI, HpaI, KpnI, MluI, NheI, PmeI, Sall, SrfI, SwaI, XbaI, XcmI, XhoI

Sequence

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cacaaggcta cttccctgat tagcagaact acacaccagg
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Component Fragments