

General Description

DNA pHAGE2-EF1aFull-Oct4F2AKlf4-IRES-Sox2E2AcMyc-W-loxp
Entire molecule length: 12520 bp

Standard Fields

User Fields

Author(s)

Author

Gustavo Mostoslavsky

Original author

Gustavo Mostoslavsky

References

Comments

Feature Map

CDS (7 total)

HIV gag

Start: 790 End: 1144

HIV gag sequence from 336bp to 688bp (Clone to PvuII site in HIV and filled in ClaI digest to

env

Start: 1156 End: 2013

HIV env sequence from bp7168 to 8025 (contains RRE) Cloned from BglII to BamHI out of HIV

Oct4

Start: 3412 End: 4467

Klf4

Start: 4543 End: 5967

Sox2

Start: 6555 End: 7511

cMyc

Start: 7581 End: 8900

AMPr

Start: 10134 End: 10973

Ampicillin Resistance Gene

Original Location Description:

6153..6992

Qualifiers:

/gene="AMPr"

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

Intron (1 total)

Intron

Start: 2454 End: 3045

LTR (1 total)

5' LTR

Start: 1 End: 636
Long Terminal Repeat
Original Location Description:
1..636
Qualifiers:
/gene="LTR"

Misc. Binding Site (1 total)

loxp

Start: 9632 End: 9665

Misc. Feature (26 total)

HIV U3

Start: 1 End: 454
Full Length HIV U3

HIV R

Start: 455 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
HIV gag sequence 351bp to 688bp

Stop Codon

Start: 853 End: 855
Stop codon in gag put in frame by ClaI fill in

RRE

Start: 1245 End: 1593
Rev Responsive Element according to Belasco in Molecular Cell, Vol. 7, 603-614, March, 2001

RRE

Start: 1303 End: 1536
Rev Responsive Element
Original Location Description:
1303..1536
Qualifiers:
/gene="rre"
/product="minimal RRE"

Junk

Start: 2014 End: 2027
Linker Junk Sequence probably left over from cloning

HIV cpPu (Trip)

Start: 2034 End: 2211
Central Poly Purine Track
Original Location Description:
2034..2211
Qualifiers:
/gene="cppt"

Human EF1a Promoter

Start: 2218 End: 3402

GSG bridge

Start: 4468 End: 4476

F2A

Start: 4477 End: 4542

IRES

Start: 5974 End: 6551

GSG bridge

Start: 7512 End: 7520

E2A

Start: 7521 End: 7580

WPRE

Start: 8913 End: 9504
Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element
Feature extends beyond the specified end point
Original Location Description:
3563..4154
Qualifiers:
/gene="WPRE"

HIV nef

Start: 9509 End: 9610
HIV nef

HIV PPT

Start: 9559 End: 9573
PolyPurine Tract

dU3

Start: 9576 End: 9612
1st Half of deleted U3

dU3

Start: 9680 End: 9698
2nd Half of deleted U3

HIV R

Start: 9699 End: 9795

HIV U5

Start: 9796 End: 9878
HIV U5

SV40 ori

Start: 11972 End: 12095
Original Location Description:
7991..8114
Qualifiers:

/gene="SV40 ori"

PolyA Signal (1 total)

G-T cluster

Start: 570 End: 577

GT Cluster shown to be necessary for Poly-A site activity by Cullen in J Virol. 1989 January;

PolyA Site (1 total)

pA Site

Start: 550 End: 550

Exact base to which Poly-A is added

Primer Binding Site (27 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 5' IRES

Start: 5990 End: 6010

pHAGE 3' CDS 1-1 (Binds IRES)

Start: 5998 End: 6018 (Complementary)

pHAGE 5' CDS 2-1 Start (Binds IRES)

Start: 6254 End: 6272

pHAGE 3' CDS 1-2 (Binds IRES)

Start: 6254 End: 6272 (Complementary)

pHAGE 3' CDS

Start: 8941 End: 8975 (Complementary)

pHAGE-A3550-3050

Start: 9019 End: 9042 (Complementary)

pHAGE-S4001-4500

Start: 9172 End: 9192

pHAGE-S6001-6500

Start: 9910 End: 9932

pHAGE-A6050-5550

Start: 10183 End: 10206 (Complementary)

pHAGE-S6501-7000

Start: 10383 End: 10407

pHAGE-A6550-6050

Start: 10652 End: 10675 (Complementary)

pHAGE-S7001-7500

Start: 10903 End: 10927

pHAGE-A7050-6550

Start: 11163 End: 11184 (Complementary)

pHAGE-S7501-8000

Start: 11407 End: 11431

pHAGE-A7550-7050

Start: 11674 End: 11695 (Complementary)

pHAGE-S8001-8500

Start: 11910 End: 11929

pHAGE-A8050-7550

Start: 12150 End: 12176 (Complementary)

pHAGE-S1-500

Start: 12415 End: 12443

Promoter Prokaryotic (1 total)

P-Bla

Start: 10081 End: 10115

Beta Lactamase Promoter

Replication Origin (1 total)

ORI

Start: 11202 End: 11735

Bacterial Origin of Replication

Original Location Description:

7221..7754

Qualifiers:

/gene="ORI"

/product="ColE1 origin of replication"

Splicing Signal (3 total)

5' SD 289

Start: 744 End: 745

Major 5' Splice Donor 289

SA 7925

Start: 1911 End: 1912

SA 4459

Start: 2188 End: 2189

Stem Loop (4 total)

SL-1

Start: 697 End: 731

PSI Stem loop 1

SL-2

Start: 736 End: 754
PSI Stem loop 2

SL-3

Start: 766 End: 779
PSI Stem Loop 3

SL-4

Start: 793 End: 806
PSI Stem Loop 4

5' UTR (1 total)

5' UTR

Start: 551 End: 635
HIV 5' UTR

Mutation (3 total)

Fill-in

Start: 833 End: 834
Filled in Cla Site from original HIV genome to disrupt gag polyprotein ORF

RRE Mutation

Start: 1357 End: 1357
This G is a C in the WT HIV-1 genome

EF1a Mutation

Start: 2531 End: 2531

Restriction/Methylation Map

Enzyme	# of cuts	Positions
Acc65I	3	6407 9506 9667
AfIII	4	518 2603 3971 9762
ApaLI	4	6443 9124 10232 11478
Ascl	1	9626
AvaI	16	296 1928 2269 2371 2557 3072 3189 3788 4984 5092 5489 6716 7110 7767 8432 9880
BamHI	1	3499
BclI	1	7379
BglIII	4	474 2790 9542 9718
Clal	1	8903
DraI	8	2062 2725 5966 9559 10326 11018 11037 12478
EagI	7	1145 1149 3405 4690 5120 6823 6892
EcoRI	1	2029
EcoRV	4	36 115 334 7725
EgeI	7	640 3164 4235 5298 6999 7349 7401
FseI	2	1151 2928
FspI	1	10681
HindIII	7	532 1088 1676 3517 6190 6602 9776
KpnI	3	6411 9510 9671
MscI	2	4721 6544

NaeI	4	1149 2926 7002 9429
NcoI	3	3411 4542 7277
NdeI	1	6551
NotI	1	3405
PmlI	2	291 6282
PstI	8	2540 3045 3819 4952 7178 7703 8120 9619
PvuI	1	10535
PvuII	5	436 7092 7878 8542 9537
SacI	6	492 683 3052 5115 5631 9736
SacII	5	2478 2825 6898 8082 9418
SbfI	2	8120 9619
SdaI	2	8120 9619
SfiI	1	12024
SmaI	4	4986 6718 7112 9882
SpeI	1	2213
StuI	5	2595 2638 3956 4418 12070
XcmI	3	5393 7284 7317
XhoI	1	3189
XmaI	4	4984 6716 7110 9880

No cuts: BsrGI, HpaI, MluI, NheI, PaeI, PmeI, Sall, SrfI, SwaI, XbaI

Sequence

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Component Fragments