



General Description

DNA pHAGE EF1a1-a1AT-W
Entire molecule length: 8285 bp

Standard Fields

User Fields

Author(s)

Author

Original author

Darrell Kotton
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References

Comments

EF1a1 promotor cloned into CMV-AAT-W by Not1/SpeI ligation by LWK on 8/05.
EF1a1 promotor including both junctions confirmed by sequencing.

Feature Map

CDS (2 total)

human a1ATT CDS

Start: 3411 End: 4664

AMPr

Start: 5899 End: 6738

Ampicillin Resistance Gene

Original Location Description:

6153..6992

Qualifiers:

/gene="AMPr"

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

Intron (1 total)

Intron

Start: 2454 End: 3045

Human EF1a Intron

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"

Human EF1a Promoter

Start: 2218 End: 3402
Human Elongation Factor 1 alpha promoter

WPRE

Start: 4674 End: 5265
Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element
Original Location Description:
3563..4154
Qualifiers:
/gene="WPRE"

HIV nef

Start: 5271 End: 5359
HIV nef

HIV PPT

Start: 5321 End: 5335
Polypurine Tract (Not degraded by RNAseH so it can serve as a primer for + strand DNA

dU3 LTR

Start: 5336 End: 5571
Deleted U3 Long-Terminal Repeat
Original Location Description:
4225..4460
Qualifiers:
/gene="dU3 LTR"

HIV R

Start: 5373 End: 5488
HIV Repeat Region

HIV U5

Start: 5489 End: 5571
HIV U5

3' Flank

Start: 5572 End: 5642
Chromosomal 3' Flanking Sequence carried over from original HIV integration site

SV40 ori

Start: 7737 End: 7860
Original Location Description:
7991..8114
Qualifiers:
/gene="SV40 ori"

Modified Base (1 total)

Difference from 5' LTR
Start: 5384 End: 5384
This T is a C in the 5' LTR

Primer Binding Site (26 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)
oligo #96
Start: 3503 End: 3527
antisense
pHAGE 3' CDS

Start: 4702 End: 4736 (Complementary)
pHAGE-A3550-3050
Start: 4780 End: 4803 (Complementary)
pHAGE-S4001-4500
Start: 4933 End: 4953
pHAGE-A4050-3550
Start: 5261 End: 5283 (Complementary)
pHAGE-S4501-5000
Start: 5569 End: 5600
pHAGE-S6001-6500
Start: 5675 End: 5697
pHAGE-A6050-5550
Start: 5948 End: 5971 (Complementary)
pHAGE-S6501-7000
Start: 6148 End: 6172
pHAGE-A6550-6050
Start: 6417 End: 6440 (Complementary)
pHAGE-S7001-7500
Start: 6668 End: 6692
pHAGE-A7050-6550
Start: 6928 End: 6949 (Complementary)
pHAGE-S7501-8000
Start: 7172 End: 7196
pHAGE-A7550-7050
Start: 7439 End: 7460 (Complementary)
pHAGE-S8001-8500
Start: 7675 End: 7694
pHAGE-A8050-7550
Start: 7915 End: 7941 (Complementary)
pHAGE-S1-500
Start: 8180 End: 8208

Promoter Prokaryotic (1 total)

P-Bla

Start: 5846 End: 5880
Beta Lactamase Promoter

Replication Origin (1 total)

ORI

Start: 6967 End: 7500
Bacterial Origin of Replication
Original Location Description:
7221..7754
Qualifiers:
/gene="ORI"
/product="ColE1 origin of replication"

Mutation (1 total)

EF1a Mutation

Start: 2531 End: 2531

Missing GCCC according to Genbank and Celera sequences

Restriction/Methylation Map

Enzyme	# of cuts	Positions
AfIII	3	518 2603 5455
AgeI	1	2302
ApaLI	3	4885 5997 7243
AvaI	9	296 1928 2269 2371 2557 3072 3189 4567 5644
BamHI	1	3486
BclI	1	3531
BglII	5	474 2790 5304 5370 5411
BssHII	1	712
DraI	8	2062 2725 4053 5321 6091 6783 6802 8243
EagI	3	1145 1149 3405
EcoRI	1	2029
EcoRV	4	36 115 334 4293
FseI	2	1151 2928
FspI	1	6446
HindIII	4	532 1088 1676 5469
NaeI	3	1149 2926 5190
NotI	1	3405
PacI	1	5654
PmlI	2	291 4110
PstI	2	2540 3045
PvuI	1	6300
PvuII	5	436 3603 3816 4193 5299
SacI	4	492 683 3052 5429
SacII	3	2478 2825 5179
SfiI	1	7789
SmaI	1	5646
SpeI	1	2213
StuI	4	2595 2638 3452 7835
XhoI	1	3189
XmaI	1	5644

No cuts: Acc65I, AfeI, AscI, BsrGI, ClaI, EgeI, HpaI, KpnI, MluI, MscI, NcoI, NdeI, NheI, PmeI, Sall,

Sequence

```
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cacaaggcta cttccctgat tagcagaact acacaccagg
101 gccaggggtc agatataccac tgacctttgg atggtgctac aagctagtac cagttgagcc
agataaggta gaagaggcca ataaaggaga gaacaccagc
201 ttgttacacc ctgtgagcct gcatgggatg gatgaccgg agagagaagt gttagagtgg
aggtttgaca gccgcctagc atttcatcac gtggcccag
301 agctgcatcc ggagtacttc aagaactgct gatatacgagc ttgctacaag ggactttccg
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 accccatacc ctattaccac tgccaattac ctgtggtttc
 8201 atttactcta aacctgtgat tcctctgaat tttttcatt ttaaagaat tgtatTTgtt

Component Fragments

#1: FRAGMENT of p201 pHAGE CMV-a1AT-W

parent position: from 2805 to 2212
 length: 7093
 molecule position: from 3405 to 2212

Left Terminus

NotI site #1

Right Terminus

SpeI site #1

#2: FRAGMENT of p302 pHAGE EF1a long-GFP-W

parent position: from 2213 to 3404
 length: 1192
 molecule position: from 2213 to 3404

Left Terminus

SpeI site #1

Right Terminus

NotI site #1