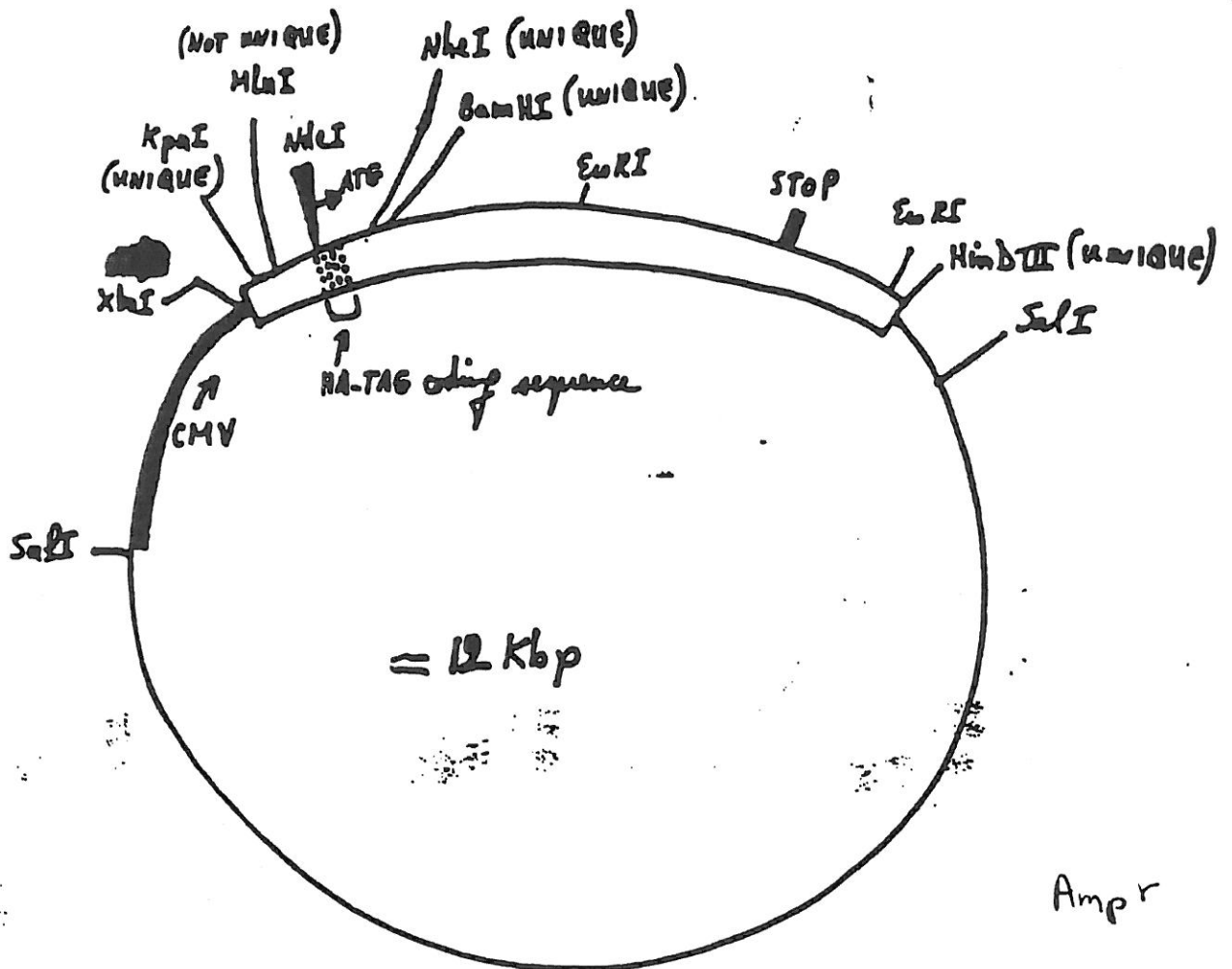


pMCL ⊕ HA-tagged MAPKK / CMV promoter: (2 SalI sites)



This construct encodes for 26 additional amino-acids at the NH₂-terminus of MAPKK.

We have various mutants in this construct.

Sunday 8/15/93

pCEP4 Lerner → pMCL1

Dear ~~Shui-chan~~:

We sequenced the region of pCEP-4/Lerner that encompasses the Lerner encoding sequence. We thought you might be interested in it as it is slightly different from the sequence you gave us. The difference is upstream from the coding region.

Primer sequence: 5'-AGG CGT GTA CGG TGG GAG-3' (in pCEP-4)

Sequence: 5'-TTTAGTGAACCGTCAGATC ^{XbaI} TCTAGA ^{KpnI} AGCTG ^{MluI} GGTACC ACGCGT _{456p}

^{NdeI} CATATG ^{LERNER Sequence} GCTTACCCATACGATGTTCCAGATTAC ^{NheI} GCTAGC

^{XhoI}
GGCCG CTCGAG-3'

We have modified the vector as follows:

pCEP-4/Lerner was digested with NheI and BamHI (the BamHI site is downstream of the XhoI site). We introduced a new insert that preserved the NheI site, followed by the sequence 5'-CTG-3', followed by a new (and unique) BamHI site, then the sequence 5'-GTGTGTGTA-3', and a PvuII site, followed by a HindIII site. The original BamHI site in pCEP-4 has been knocked out in the process. The new construct has the following organization:

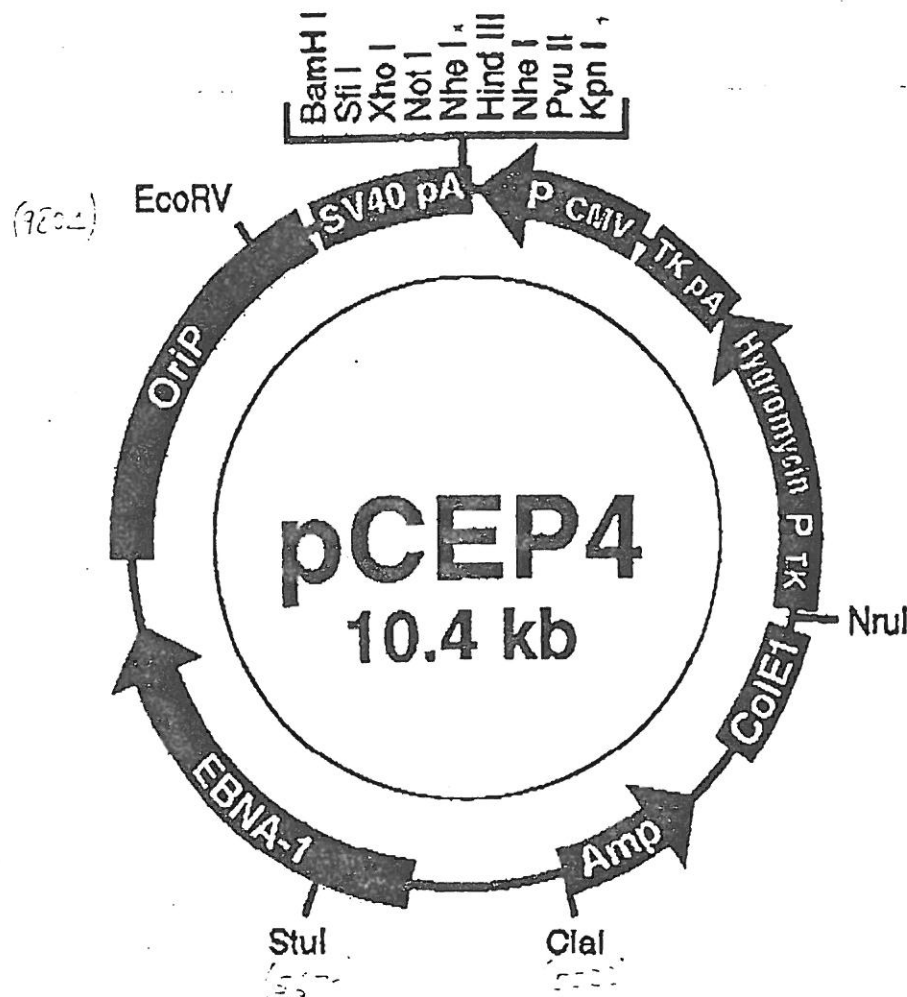
Lerner tag coding region/NheI/5'-CTG-3'/BamHI/9bp/PvuII/HindIII
The modification has rearranged the order of the restriction sites present in the original vector; BamHI is now upstream of PvuII and HindIII.

You are welcome to have the modified vector.

Common to all pCEP4
10410 base pairs

SV40 Poly A signal: bases 7-405
Multiple Cloning Site: bases 406-463
CMV Promotor: bases 467-1311
TK Poly A signal: bases 1473-1843
Hygromycin gene: bases 1844-2893
TK Promotor: bases 2894-3136
Ampicillin resistance/pUC origin: bases 3635-5526
EBNA-1 gene: bases 5533-8113
Ori P: bases 8142-10078

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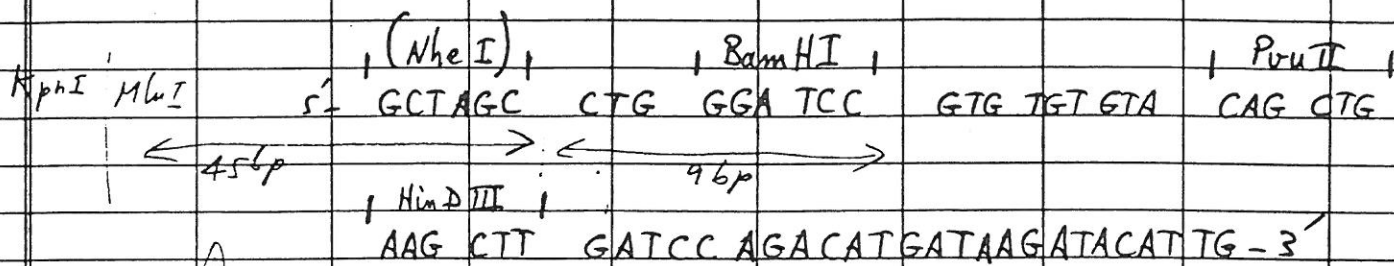
V2.5-130208sa

For Technical Service Call
1-800-955-6288

8/15/93

MCL-1 Modification: (See gel #1027)

Same as pCEP-A/Lerner, up to Nhe I site (still preserved)



Total 54

10	20	30	40	50	60
TCTAGAGTCG	ACCGGTCATG	GCTGCGCCCC	GACACCCGCC	AACACCCGCT	GACGCGCCCT
AGATCTCAGC	TGGCCAGTAC	CGACGCGGGG	CTGTGGGCGG	TTGTGGGCGA	CTGCGCGGGA
70	80	90	100	110	120
GACGGGCTTG	TCTGCTCCCG	GCATCCGCTT	ACAGACAAGC	TGTGACCGTC	TCCGGGAGCT
CTGCCCCAAC	AGACGAGGGC	CCTAGCCCCA	TCTCTCTTCC	ACACTGGCAG	AGCCCCCTCA
130	140	150	160	170	180
GCATGTGTCA	GAGGTTTTCA	CGGTTCATAC	CGAAACCGGC	GAGGCAGCCG	GATCATAATC
CGTACACAGT	CTCCAAAAGT	GGCAGTAGTG	GCTTTGCGCG	CTCCGTCGGC	CTAGTATTAG
190	200	210	220	230	240
AGCCATACCA	CATTTGTAGA	GGTTTACTTT	GCTTTAAAAA	ACCTCCCCAC	CTCCCCCTGA
TCGGTATGGT	GTAAACATCT	CCAAAATGAA	CGAAATTTTT	TGGAGGGGTG	GAGGGGGACT
250	260	270	280	290	300
ACCTGAAACA	TAAAAATGAAT	GCAATTGTTG	TTGTTAACTT	GTTTATTGCA	GCTTATAATG
TGGACTTTGT	ATTTTACTTA	CGTTAACAA	AACAATTGAA	CAATAACGT	CGAATATTAC
310	320	330	340	350	360
GTTACAAATA	AAGCAATAGC	ATCACAAATT	TCACAAATAA	AGCATTTTTT	TCACTGCAIT
CAATGTTTTAT	TTGGTTATCG	TAGTGTTTAA	ACTGTTTTAT	TCGTAAAAAA	AGTGACGTAA
370	380	390	400	410	420
CTAGTTGTGG	TTTGTCCTAA	CTCATCAATG	TATCTTATCA	TGTCTGGATC	CGGCTTTGCC
GATCAACACC	AAACAGGTTT	GAGTAGTTAC	ATAGAATAGT	ACAGACCTAG	GCCGGAACGG
GGCCTCGAGC	GGCCGCTAGC	AAGCTTGCTA	GCAGCTGGTA	COCAGCTTCT	AGAGATCTGA
CCGGAGCTCG	CCGGCGATCG	TTGGAACGAT	CGTCGACCAT	GGGTGGAAGA	TCTCTAGACT
490	500	510	520	530	540
CGGTTCACTA	AACGAGCTCT	GCTTATATAG	ACCTCCCACC	GTACACGCCT	ACCGCCCAIT
GCCAAGTGAT	TTGCTCGAGA	CGAATATATC	TGGAGGGTGG	CATGTGCGGA	TGGCGGGTAA
550	560	570	580	590	600
TGCGTCAACG	GGGCGGGGTT	ATTACGACAT	TTTGAAAGT	CCCGTTGATT	TTGGTGCCAA
ACGCAGTTGC	CCCGCCCCAA	TAATGCTGTA	AAACCTTTCA	GGGCAACTAA	AACCACGGTT
610	620	630	640	650	660
AACAAACTCC	CATTGACGTC	AATGGGGTGG	AGACTTGGAA	ATCCCCGTGA	GTCAAACCGC
TTGTTTGAGG	GTAACGTCAG	TTACCCCACC	TCTGAACCTT	TAGGGGCACT	CAGTTTGGCG
670	680	690	700	710	720
TATCCACGCC	CATTGGTGTA	CTGCCAAAAC	CGCATCACCA	TGGTAATAGC	GATGACTAAT
ATAGGTGCGG	GTAACCACAT	GACGGTTTTG	GCGTAGTGGT	ACCATTATCG	CTACTGATTA
730	740	750	760	770	780
ACGTAGATGT	ACTGCCAAGT	AGGAAAGTCC	CGTAAGGTCA	TGTACTGGGC	ATAATGCCAG
TGCATCTACA	TGACGGTTCA	TCCFTTTCAGG	GCAITTCAGT	ACATGACCCG	TATTACGGTC
790	800	810	820	830	840
GCGGGCCAIT	TACCGTCAIT	GACGTCAATA	GGGGGCGGAC	TTGGCATATG	ATACACTTGA
CGCCCCGTAA	ATGGCAGTAA	CTGCAGTTAT	CCCCCGCCTG	AACCGTATAC	TATGTGAACT
850	860	870	880	890	900
TGTACTGCCA	AGTGGGCAGT	TTACCGTAAA	TACTCCACCC	ATTGACGTCA	ATGGAAGTTC
ACATGACGGT	TCACCCGTCA	AATGGCATTT	ATGAGGTGGG	TAACTGCAGT	TACCTTTCAG
910	920	930	940	950	960
CCTATTGGCG	TTACTATGGG	AACATACGTC	ATTATTGACG	TCAATGGGCG	GGGGTGGTTG
GGATAACCGC	AATGATACCC	TTGTATGCAG	TAATAACTGC	AGTTACCCGC	CCCCAGCAAC

Before Modification