

Plasmid Name: pMM288

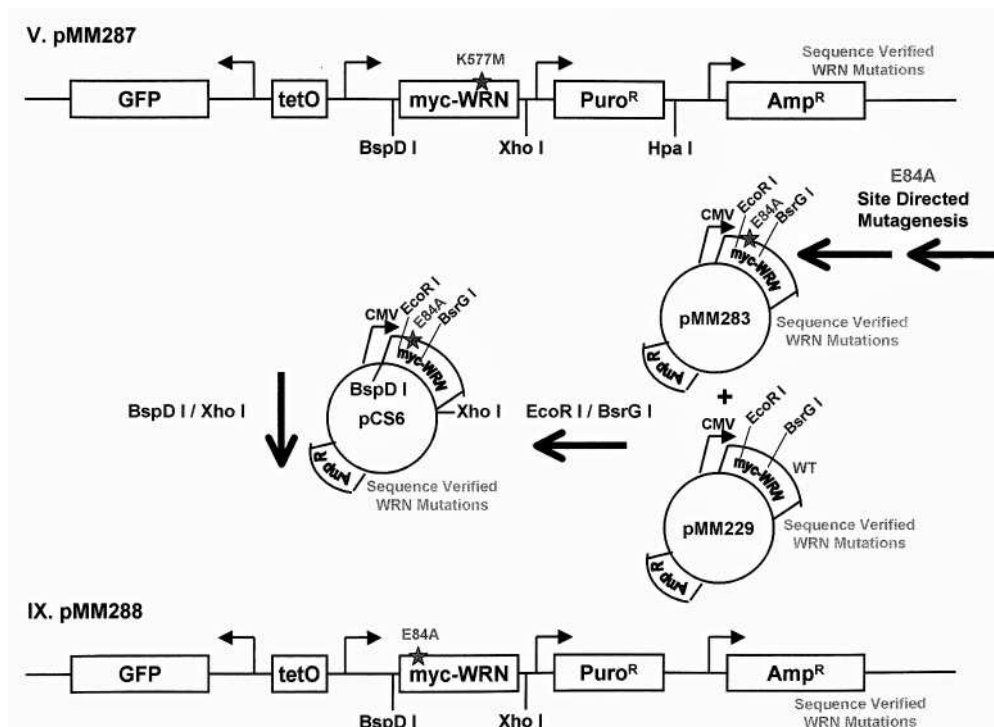
Aliases: none known

Length: 11707 bp

Constructed by: Mike Moser/Cristina Swanson

Last updated: 17 August 2009

Description and application: This is a mammalian expression vector for human WRN protein that lacks exonuclease activity by virtual of an E84A substitution. It was constructed from pBI, a Clontech plasmid with a bi-directional promoter + TRE backbone, modified by insertion of a polylinker containing BspD1 and XhoI sites and a myc-WRN E84A ORF – created by making E84A (A>C) mutation in pMM290 sequence [NB this is not how the plasmid was originally constructed – see plasmid construction outline below.]



NB: the WRN sequence in this plasmid contains a number of variations from the WT WRN sequence (NM_000553.4). These polymorphisms are either silent or do not to alter the function of the WRN protein. *The A>G nucleotide change at position 235 was introduced into the WRN sequence during the cloning to generate myc epitope-tagged WRN. The subsequent amino acid change does not appear to have any functional consequence on the WRN protein.

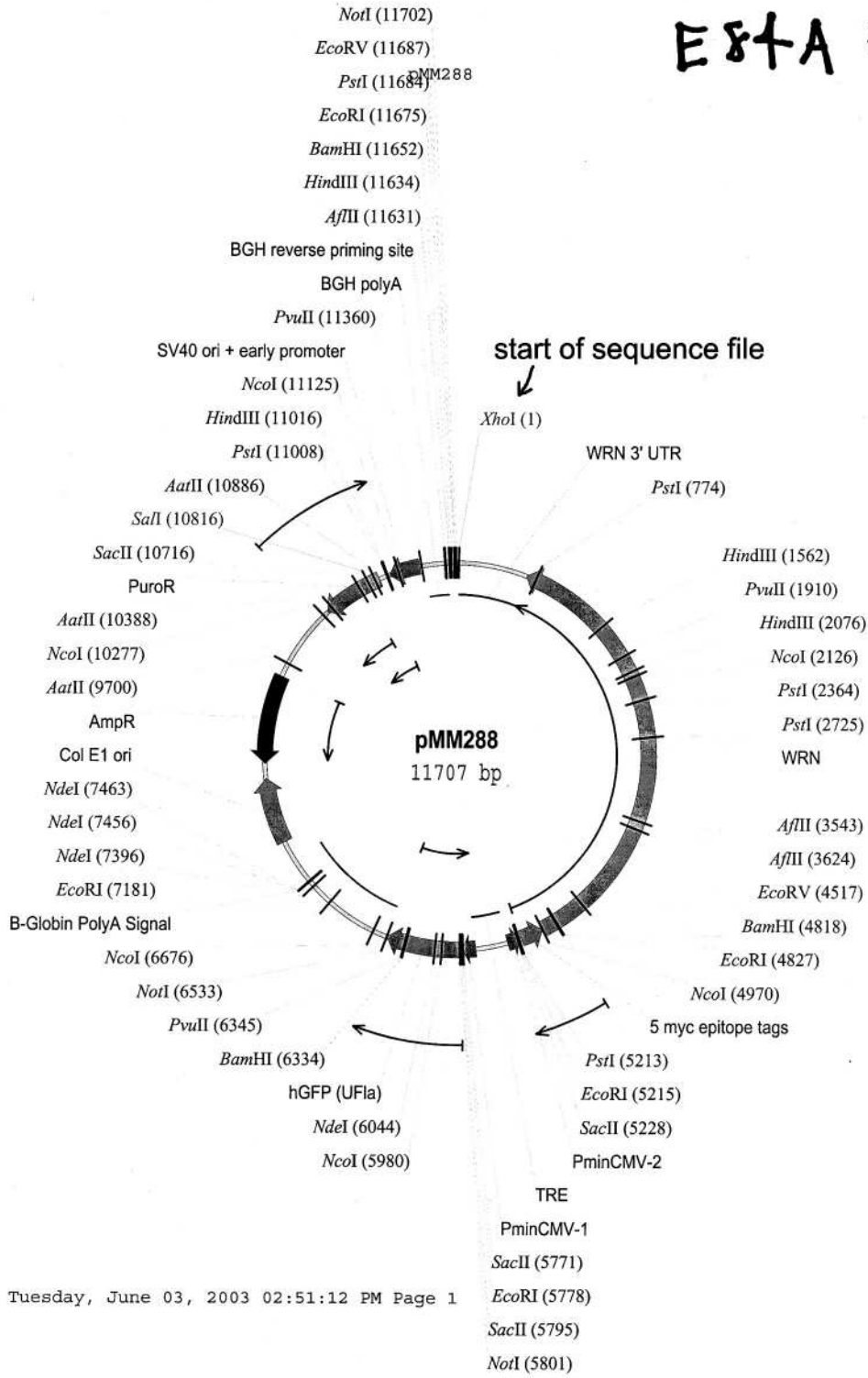
| Nucleotide position in cDNA | Exon | Nucleotide change | Amino acid number | Amino acid change |
|-----------------------------|------|-------------------|-------------------|-------------------|
| 235* | 2 | A>G | 2 | Ser>Gly |
| 270 | 2 | G>A | 13 | Arg>Arg |
| 501 | 4 | T>C | 90 | Asn>Asn |
| 678 | 5 | A>T | 149 | Arg>Arg |
| 1386 | 9 | A>G | 385 | Glu>Glu |
| 2592 | 20 | T>G | 787 | Leu>Leu |
| 3453 | 26 | T>G | 1074 | Phe>Leu |
| 4314 | 34 | C>T | 1361 | Ser>Ser |

Selections: AmpR, PuroR

[continue to next page]

Map:

ESTA WRN



Feature table:

| | |
|-----------|----------------------|
| WRN | 677 – 5191, reverse |
| PminCMV's | 5796-6527, forward |
| AmpR | 8707-9564, reverse |
| PurOR | 10332-10999, reverse |

MCS/polylinker sequence:

Experimental verification: YES

by restriction mapping: Y

DNA sequencing: Y

Western or other expression check/other [list]: Western

Location: wet/frozen/glycerol stock/transfected cells/other [list]

Frozen stock in P-374

Glycerol in N-314

Source of sequence: VectorNTI collection

Sequence:

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