

**Plasmid Name:** pMM289

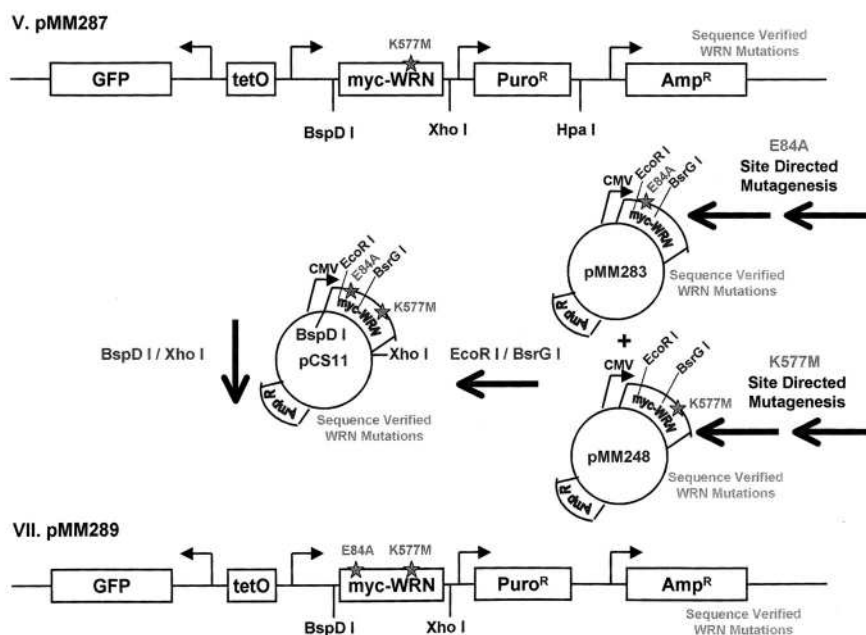
**Aliases:** none known

**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 or helicase activity by virtue of E84A (exo) and K577M (helicase) inactivating residue substitutions. 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 followed by insertion of myc-WRN E84A K577M – created by making E84A (A>C) mutation in pMM287 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 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.

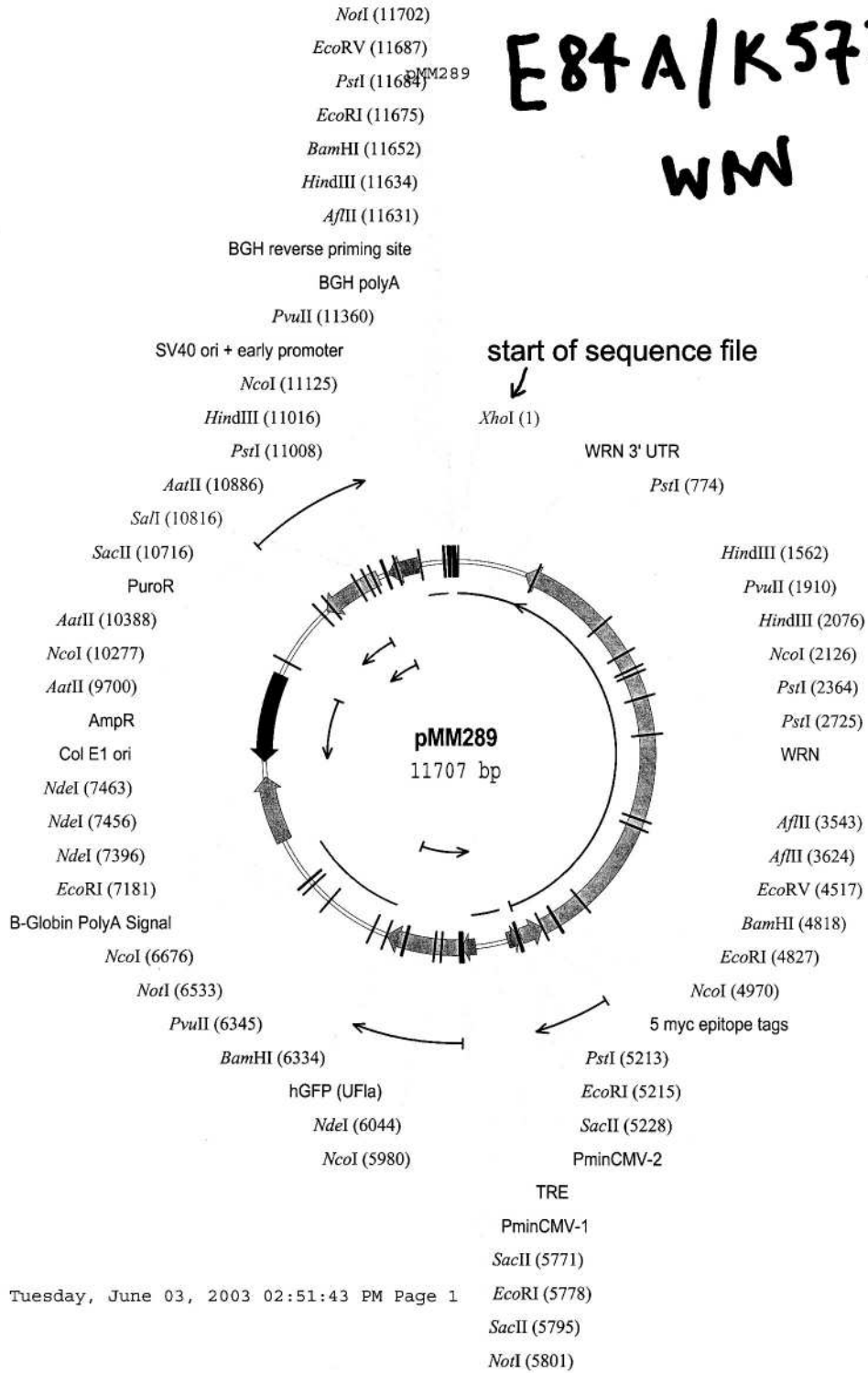
Nucleotide position in	Exon	Nucleotide change	Amino acid number	Amino acid
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cDNA				change
235*	2	A>G	2	Ser>Gly
270	2	G>A	13	Arg>Arg
501	4	T>C	90	Asn>Asn
678	5	A>T	149	Arg>Arg
1386	9	A>G	385	Glu>Glu
2592	20	T>G	787	Leu>Leu
3453	26	T>G	1074	Phe>Leu
4314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

**Selections:** AmpR, PuroR

[continue to next page]

**Map:**



**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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