Plasmid Name: pMM290

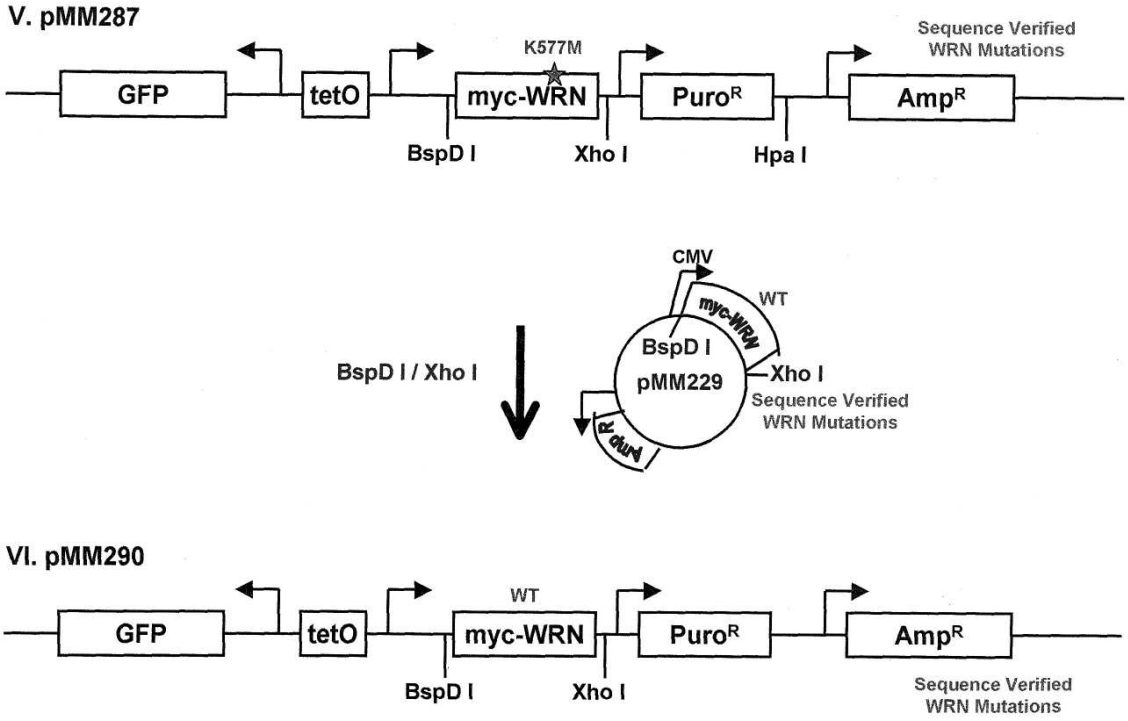
Aliases: none known

Length: 11707 bp

Constructed by: Mike Moser/Cristina Swanson

Last updated: 17 August 2009

Description and application: This is a mammalian expression vector for catalytically active human WRN protein. 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 WT. Vector backbone is pMM286, myc-WRN WT came from DspD1-XhoI fragment from pMM229. [NB this is not how the plasmid was originally constructed – see plasmid construction outline below.]



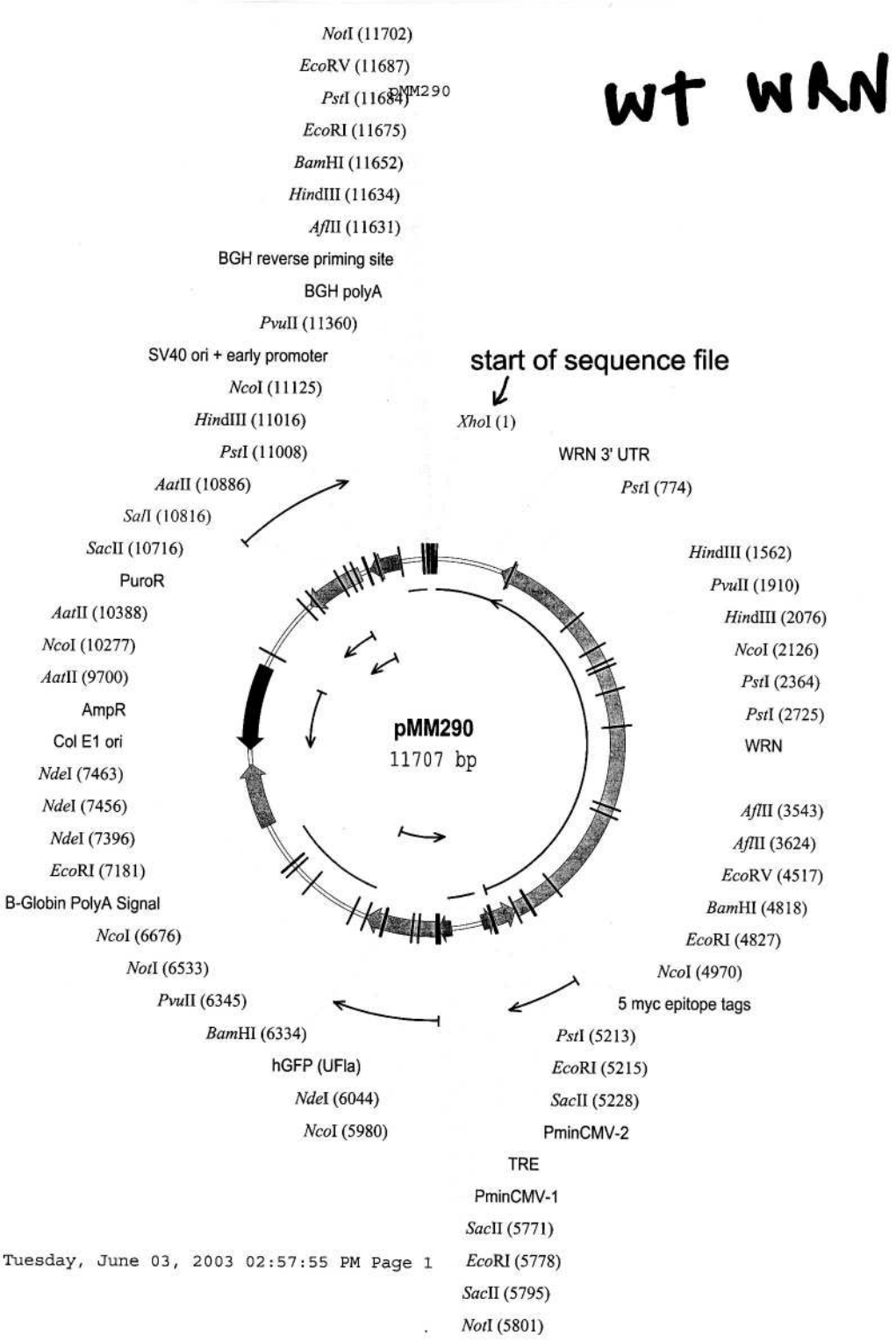
NB: the WRN sequence in this plasmid contains a number of variations from the WT WRN sequence (NM_000553.4). These polymorphisms are either silent or do not to alter the function of the WRN protein. *The A>G nucleotide change at position 235 was introduced into the WRN sequence during the cloning to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

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:


wt WRN
pMM290
NotI (11702)
EcoRV (11687)
PstI (11684)
EcoRI (11675)
BamHI (11652)
HindIII (11634)
AflII (11631)
BGH reverse priming site
BGH polyA
PvuII (11360)
SV40 ori + early promoter
NcoI (11125)
HindIII (11016)
PstI (11008)
AatII (10886)
SalI (10816)
SacII (10716)
PuroR
AatII (10388)
NcoI (10277)
AatII (9700)
AmpR
Col E1 ori
NdeI (7463)
NdeI (7456)
NdeI (7396)
EcoRI (7181)
B-Globin PolyA Signal
NcoI (6676)
NotI (6533)
PvuII (6345)
BamHI (6334)
hGFP (UFla)
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NcoI (5980)
start of sequence file
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WRN 3' UTR
PstI (774)
HindIII (1562)
PvuII (1910)
HindIII (2076)
NcoI (2126)
PstI (2364)
PstI (2725)
WRN
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AflII (3624)
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BamHI (4818)
EcoRI (4827)
NcoI (4970)
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EcoRI (5215)
SacII (5228)
PminCMV-2
TRE
PminCMV-1
SacII (5771)
EcoRI (5778)
SacII (5795)
NotI (5801)
pMM290
11707 bp
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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: 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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