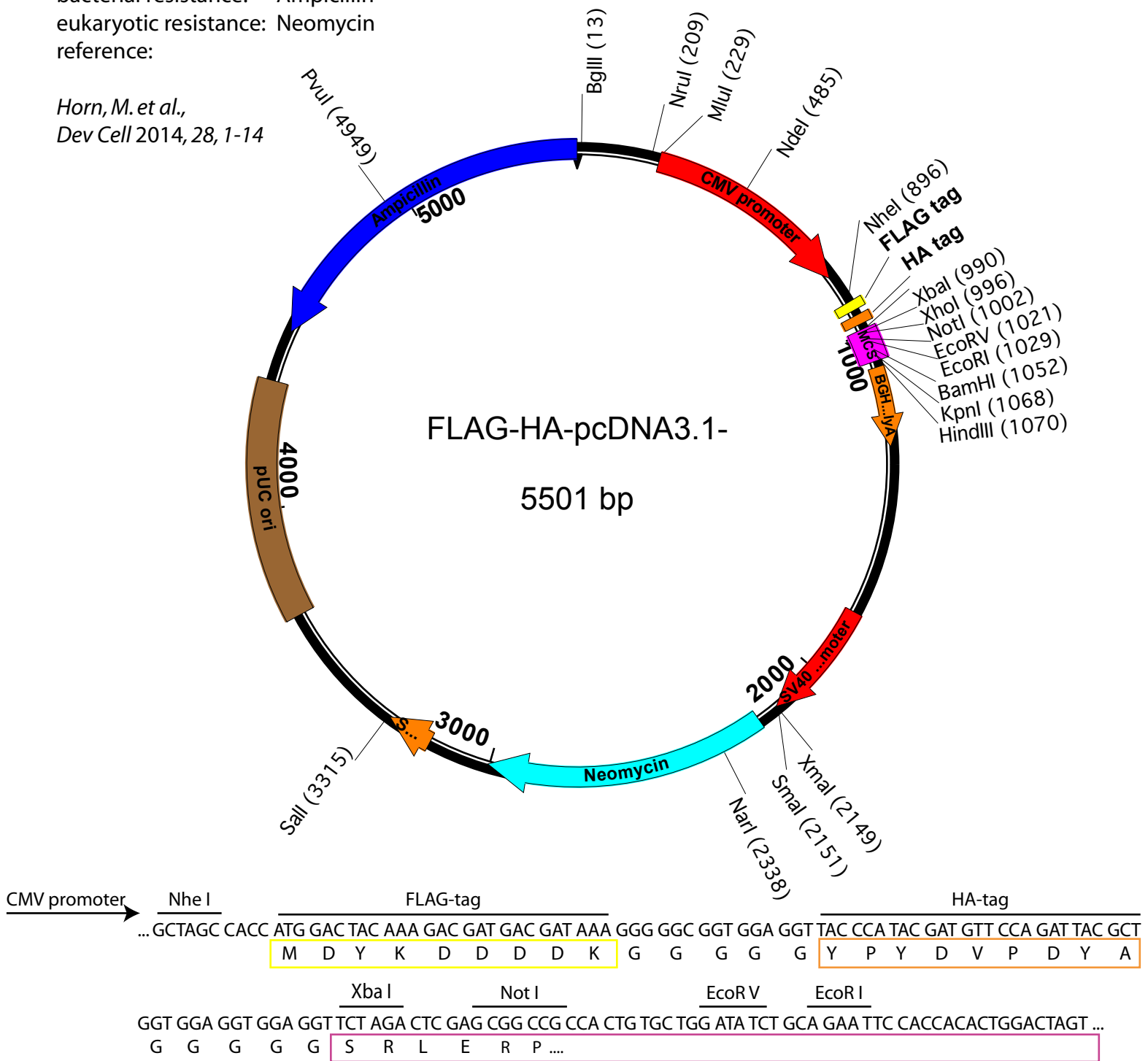


Antebi lab plasmid
 plasmid name: FLAG-HA-pcDNA3.1(-)
 constructed by: C. Geisen
 map by: C. Geisen, 11/27/13
 bacterial resistance: Ampicillin
 eukaryotic resistance: Neomycin
 reference:

Horn, M. et al.,
 Dev Cell 2014, 28, 1-14



- oligos encoding FLAG-tag sequence were inserted into pcDNA3.1- (Invitrogen) cut Nhe I / Apa I to create FLAG-pcDNA3.1-, oligos used:
 Nhe-FLAG-Apa-sense: CTAGCCACCARGGACTACAAAGACGATGACGATAAAGGGCC
 Nhe-FLAG-Apa-reverse: CTTTATCGTCATCGTCTTTGTAGTCCATGGTGG
- oligos encoding an HA-tag were inserted into FLAG-pcDNA3.1- (Invitrogen) cut Apa I / Xba I to create an intermediate FLAG-HA-pcDNA3.1-, oligos used:
 HA-insert-sense: CGGTGGAGGTTACCCATACGATGTTCAGATTACGCTGGTGGAGGTGGAGGT
 HA-insert-reverse: CTAGAACCTCCACCTCCACCAGCGTAATCTGGAACATCGTATGGGTAACCTCCACCGGGCC
- this new construct was then modified by site directed mutagenesis using primers
 FLAG-HA-2x5xGly change-sense: CGATGACGATAAAGGGggCGGTGGAGGTTACCCA
 FLAG-HA-2x5xGly change-reverse: TGGGTAACCTCCACCGccCCCTTTATCGTCATCG
- to finally create FLAG-HA-pcDNA3.1- which has two 5xGly-repeats separating the FLAG from the HA tag and the HA tag from the MCS to allow for some rotational freedom of the tags.
- to create a cDNA with N-terminal FLAG-HA-tag, clone into sites downstream of Xba I, choose proper reading frame!