Construction of pML9.7-3xHA plasmid

The plasmid pML9.7 was constructed by cloning a 9.7 kb *Sal* I genomic fragment containing the *UNI2* gene into the *Sal* I site of pBlueScript II KS (-) (see Piasecki et al., Mol. Biol. Cell 19:262-273.

To create pML9.7-3xHA , the pML9.7 plasmid was first modified by removing sequences between the *Eco*RV site and the *Xba* I site in the multi-cloning site positioned near the 3' end of the *UNI2* gene. This step removed the *Bam* HI site from the multi-cloning site. The plasmid was then digested with *Bam* HI which cleaves the *UNI2* gene at a position 13 codons upstream of the stop codon. The *Bam* HI overhang was filled in using Klenow. A blunt-ended *Nru* I/*Sma* I fragment from the p3xHA plasmid was then ligated into the blunted *Bam* HI site.

The amino acid sequence flanking the epitope tag reads: ERI – 3XHA - IQA