



Constructing HPV16 Expression Vectors with In-Fusion Cloning

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Introduction

HPV-associated head and neck squamous cell carcinomas (HNSCCs) comprise a growing number of head and neck cancer occurrences in the United States, with HPV+ tumors displaying better prognosis and chemo-radiation response. Among particular significance is HPV16, a viral subtype that has been implicated as a high risk factor for HNSCC. Previously, our group identified two distinct subgroups within HPV+ tumors based on the gene expression profile of HPV-associated genes (Figure 1). These two clusters demonstrated differential gene expression patterns and thus, differing responses to radiation treatment (Figure 2).

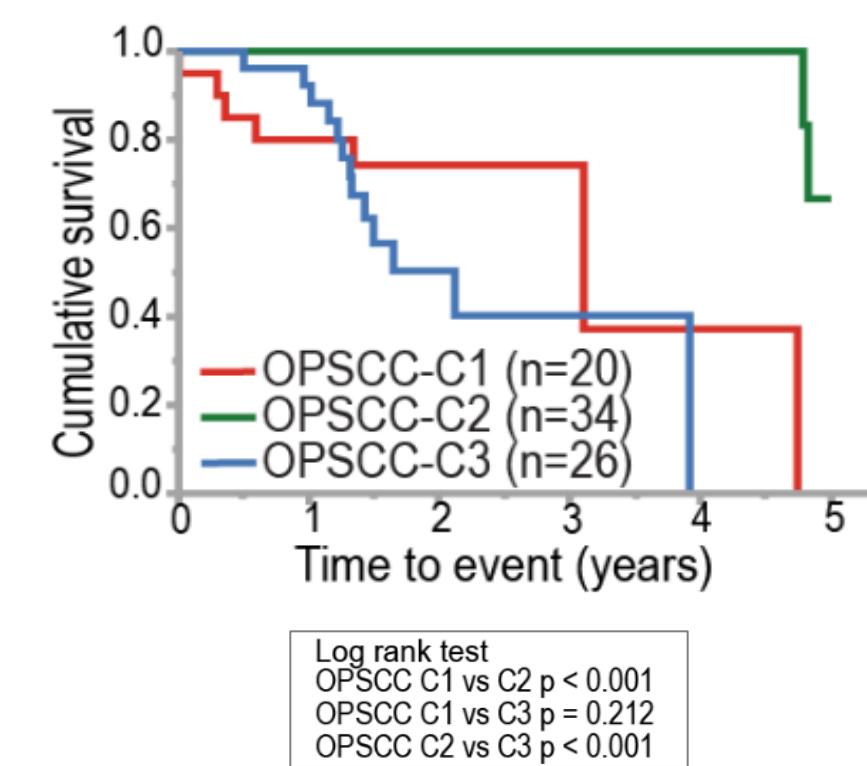


Figure 2. (Gleber-Netto, F.O. et al. Unpublished Results). Significant difference in overall 5-year survival was observed between HPV-positive OPSCC Cluster 1 and Cluster 2.

Several critical proteins encoded by the HPV genome are involved in head and neck tumorigenesis, including E6 and affiliated E6* splice products, E7, and E1^E4 (Figure 3). The E6 and E7 proteins impair the activity of tumor suppressors p53 and pRb, respectively. The spliced variant E1^E4 has been linked to the disruption of the cytoskeleton network, decreases in mitochondrial membrane potential, and apoptosis. Prior research has found that lower E1^E4 RNA expression is associated with greater resistance to radiation. Despite this correlation, it is uncertain whether the full E1^E4 protein plays a causative role in radiation sensitivity.

As part of this project, HPV16 expression vectors were created to test for phenotypes affecting the HPV prognostic signature, including radiation sensitivity, doubling times of cell lines, molecular alterations related to HPV function, and cancer drug responses. These constructs can ultimately help serve as novel tools to evaluate key genetic interactions and metabolic pathways involved in HPV+ HNSCC progression.

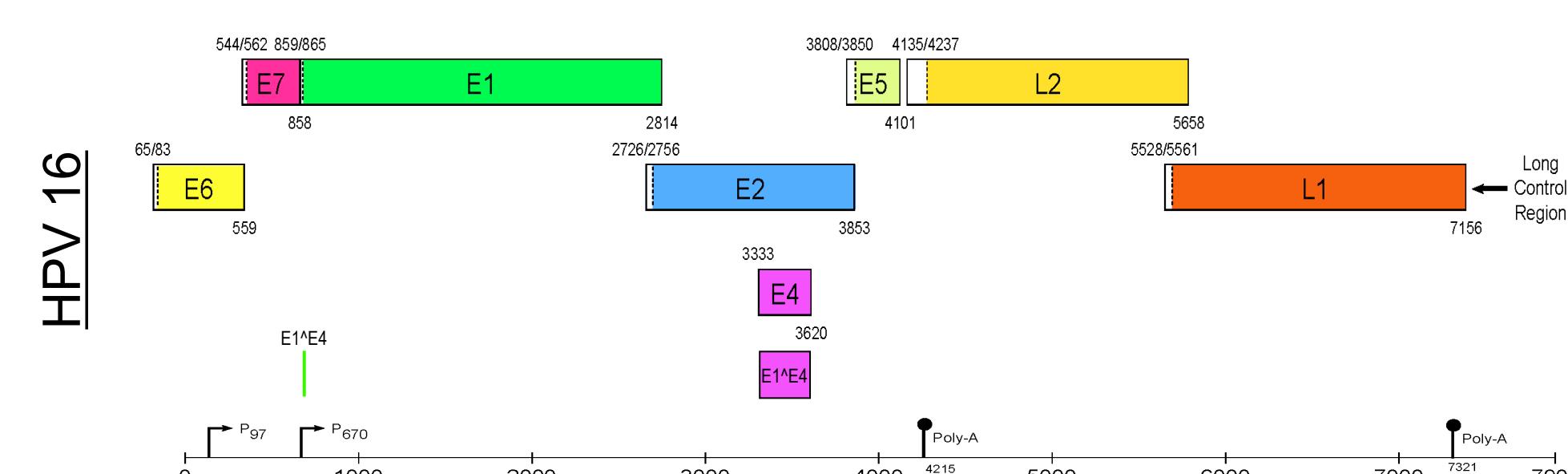
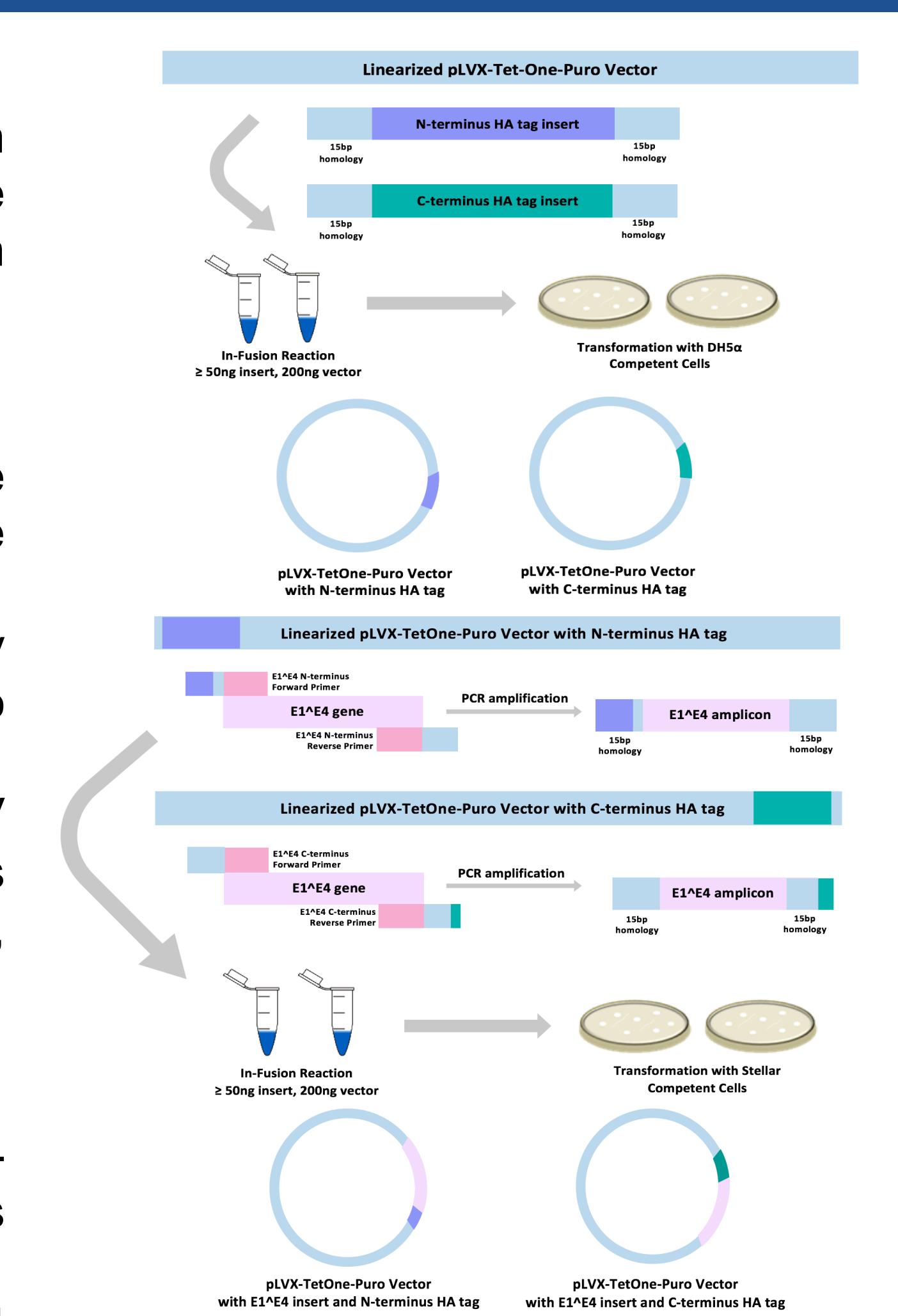


Figure 3. HPV16 transcript map from the NIH Papillomavirus Episteme database. Genes of interest include E6, E6* isoforms, E7, and the E1^E4 splice product which produces a protein comprised of the initial few amino acids of E1 combined with the E4 open reading frame.

Methods

Vector Design

- pLVX-TetOne-Puro lentiviral vector with customized HA epitope tag on either the N-terminus or C-terminus end added with In-Fusion Cloning



PCR Amplification

- Total RNA extracted from HPV16-positive HNSCC cell line UDSCC-2 to synthesize cDNA
- Primers designed to yield 15bp homology with the modified pLVX-TetOne-Puro Vector containing the HA tag
- PCR conducted to generate various HPV amplicons, including E6 and its associated splice variants, E7, E1^E4, and E6*I+E7

Cloning of HPV Amplicons

- In-Fusion Cloning to construct sequence-verified viral expression vectors containing HPV genes
- Transient transfection performed to test for RNA and protein expression

Results

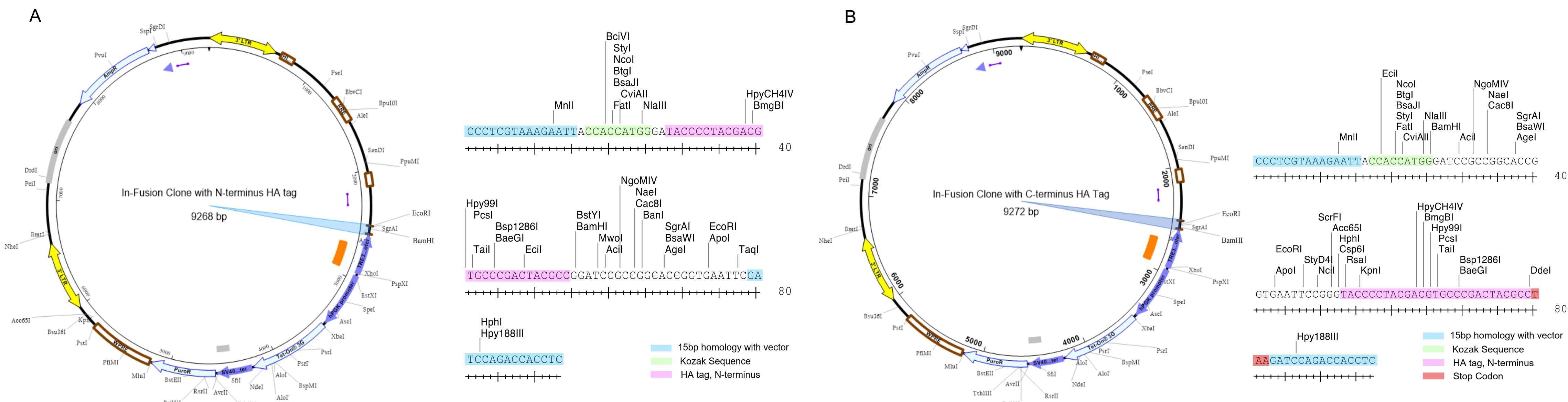


Figure 5. Modified pLVX-TetOne-Puro lentiviral vector containing the respective A) 93bp N-terminus HA tag and B) 97bp C-terminus HA tag. Shaded regions represent the annealed HA tag oligonucleotides cloned into the viral vector. Given the lack of well-defined HPV protein-specific antibodies, inclusion of the HA epitope tag within the translational open reading frame allows for general detection of the protein of interest.

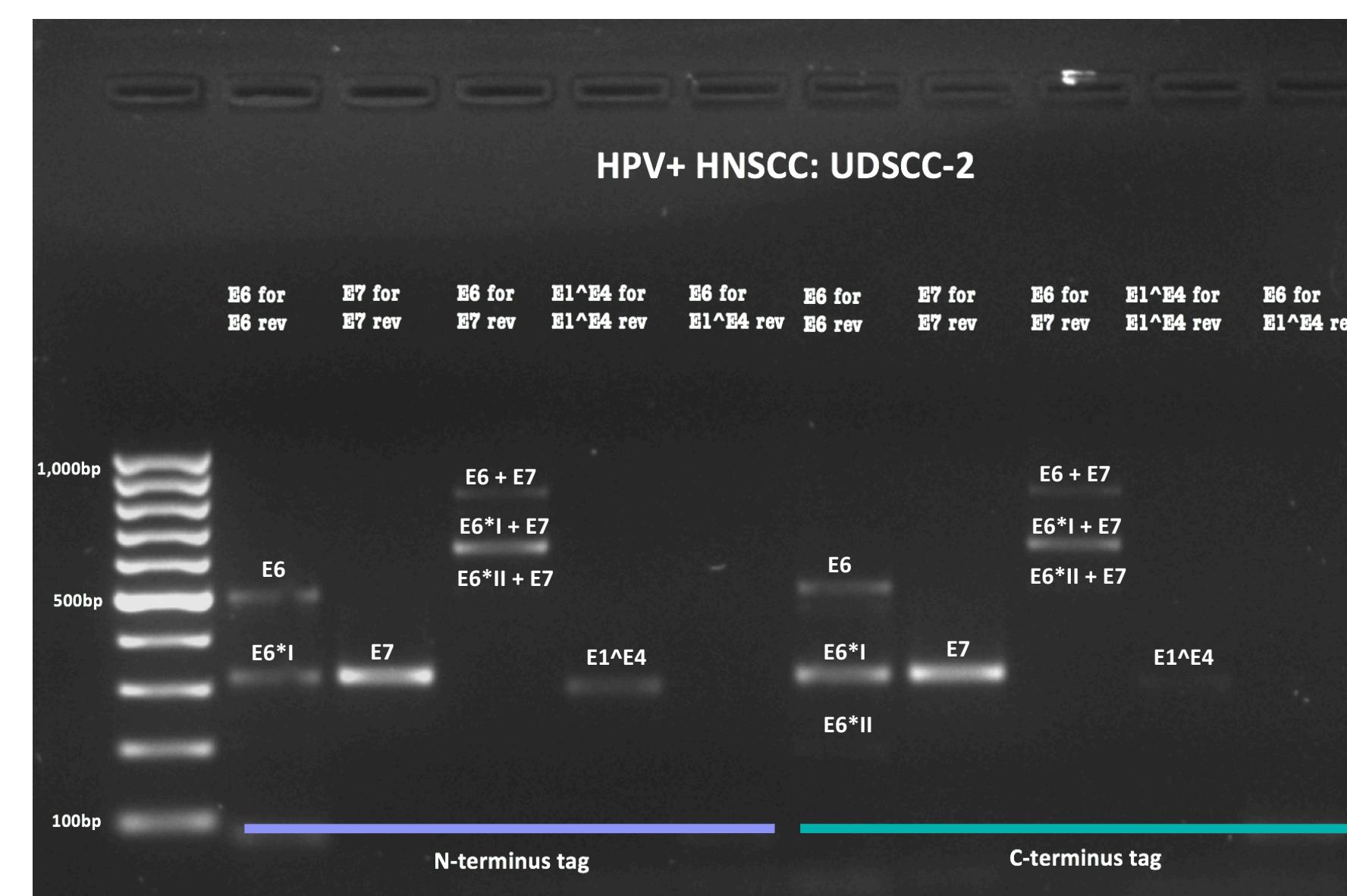


Figure 6. HPV amplicons derived from UDSCC-2 cDNA. PCR was used to amplify select HPV genes with primers exhibiting 15bp homology with either the N-terminus or C-terminus HA tag added to the pLVX-TetOne-Puro vector. Multiple bands indicate splice products associated with the E6 gene.

Transcript	Expected Sizes of PCR Products		
	Overhang	Primer	Amplicon
E6	477	31	508
E6*I	294	31	325
E6*II	177	31	208
E7	297	31	328
E1^E4	279	31	310
E6+E7	766	31	797
E6*I+E7	583	31	614
E6*II+E7	466	31	497
E6+EI^E4	750	31	781
E6*I+EI^E4	567	31	598
E6*II+EI^E4	450	31	481

Figure 6. HPV amplicons derived from UDSCC-2 cDNA. PCR was used to amplify select HPV genes with primers exhibiting 15bp homology with either the N-terminus or C-terminus HA tag added to the pLVX-TetOne-Puro vector. Multiple bands indicate splice products associated with the E6 gene.

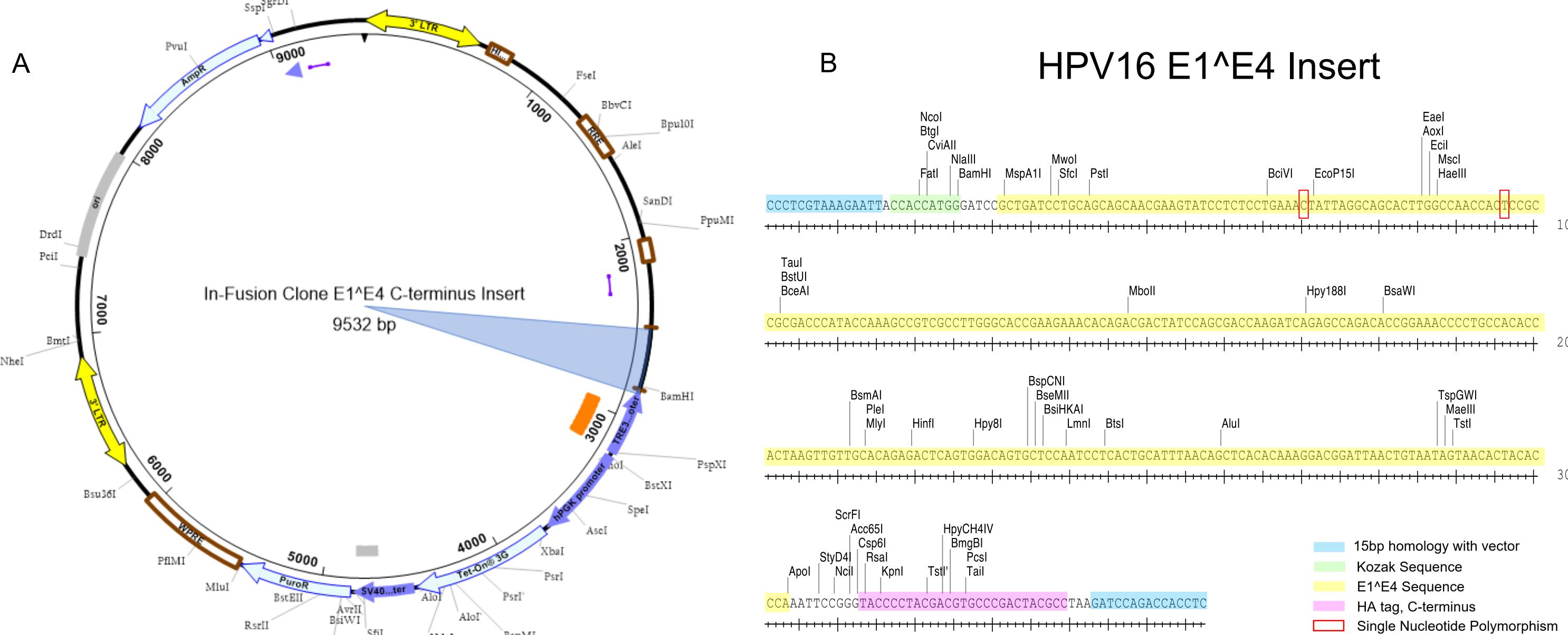


Figure 7. A) HPV16 E1^E4 gene from the UDSCC-2 cell line cloned into a modified pLVX-TetOne-Puro Vector with a C-terminus HA tag. B) SANGER sequencing of the plasmid revealed two polymorphisms when compared to the E1^E4 reference sequence from the NIH Papillomavirus Episteme database. RNA-Seq mutation results from UDSCC-2 identified the two nucleotide variations at position 3410 from C to T and position 3384 from T to C as the first and second most frequently occurring polymorphisms, respectively. No change in the amino acid sequence was observed.

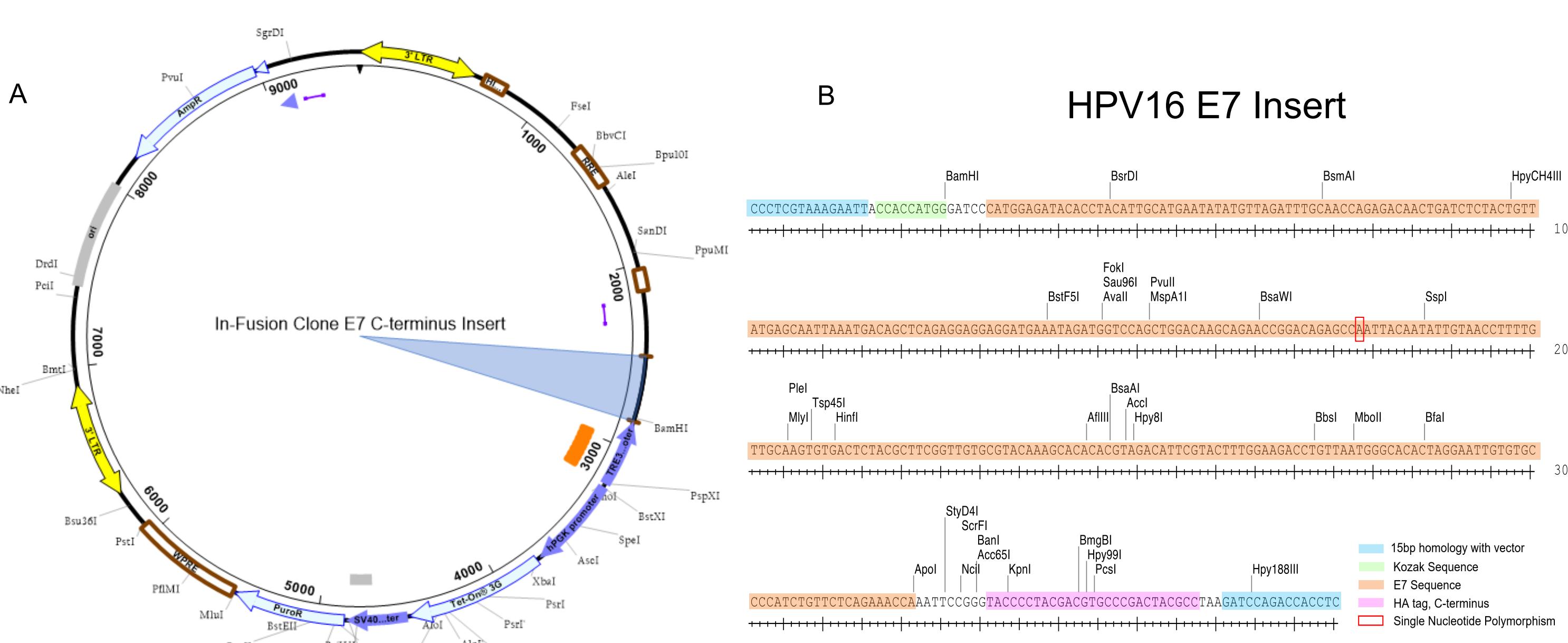


Figure 8. A) HPV16 E7 gene from the UDSCC-2 cell line cloned into a modified pLVX-TetOne-Puro Vector with a C-terminus HA tag. B) SANGER sequencing of the plasmid displayed a single polymorphism when compared to the E7 reference sequence from the NIH Papillomavirus Episteme database. The nucleotide variation from C to A at position 712, classified as the ninth most common polymorphism, resulted in the amino acid 51 change from Histidine to Asparagine.

Conclusions

- Using In-Fusion Cloning, multiple expression vectors containing HPV genes of interest were produced and sequence-verified.

In-Fusion Cloning Constructs		
HPV	Insert	HA tag
HPV16	E1^E4	N-terminus
HPV16	E1^E4	C-terminus
HPV16	E7	N-terminus
HPV16	E7	C-terminus
HPV16	E6	C-terminus
HPV16	E6*I	N-terminus

Future Directions

- Following transfection with the expression vector, virus can be created to infect HNSCC cell lines and express the proteins under an inducible doxycycline treatment.
- Upon confirmation of protein expression using the HA antibody, HPV prognostic phenotypes at the genomic, transcriptomic, and proteomic level can be examined to identify potential biomarkers and aid in classifying varied patient response to HNSCC treatment.

References

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