

2007 Avian Biosciences Center Grant Program

Final Report

Project Director: Dr. Mark S. Parcels, Associate Professor, Department of Animal and Food Sciences

Project Title: *Molecular Cloning and Characterization of a Highly-pathogenic Infectious Laryngotracheitis Virus Field Strain*

Priority Area: Poultry Health

Duration: January 1, 2008 – December 31, 2008
(funds from January 1, – June 30, 2008)

Funding Amount: \$ 25,000

Summary of Activity

The goals of this project were to:

- (1) To insert the mini-F plasmid (bacterial artificial chromosome) sequences at a deletion within the Us9 gene of the ILTV genome of a recent isolate (NC). We will attempt two different strategies for the generation of this infectious clone.
- (2) Using this infectious clone, we plan to insert a novel recombination site at an inter-genic region in the ILTV genome. This will allow for the direct introduction of foreign DNA sequences and can be used in generating complementing mutations for direct gene knock-outs.
- (3) To characterize the infectious clone, and the subsequent mutant, in comparison to the wild-type ILTV strain (NC) in vivo. We will employ an eyedrop-challenge model of 3-week-old SPF leghorns held in isolators. Infections will be compared according the duration of shed, tracheal histopathology, lesion score and the induction of antibody response.

To date, we are still working on goals 1 and 2. We have adopted the following strategy and made the following progress:

- (1) We are generating a transfer vector for the intergenic insertion of foreign sequences at a unique *loxP* site (Cre recombinase recognition site). This construction is outlined in Figure 1, below. Essentially, each arm of this vector has been cloned and its sequence determined. We have selected clones of each arm and are currently screening clones that have these arms together in the correct orientation. As each arm had been cloned

into pCR2.1 TOPO, additional restriction endonuclease sites were available for the proper directional cloning strategy. The arms of this vector are comprised of the glycoprotein C (UL44) and UL21 tegument genes. This site was chosen because the intergenic region between these genes contained two distinct transcriptional terminators, as well as > 100 nt of intergenic region for foreign sequence insertion. For foreign sequence insertion, we have generated a loxP sequence that is flanked by two poly-stop sequences. Polystop sequences are 12 nt stretches that contain stop codons in all 6 open reading frames (Figure 1D). This essentially insulates any foreign sequences inserted at the loxP from generating any proteins that would initiate within the sequences inserted at the *loxP* site, by terminating them at these sites. Inclusion of these sequences is important should any commercial development of our BAC clones become warranted.

- (2) For use as a BAC vector, we are making a derivative of pBeloBac 11, a vector that has been in the public domain for over a decade. We are inserting a selectable marker for infectious clone selection (*gpt*). This guanosyl phosphoribosyl transferase gene was amplified from pVEC-12 as a 900 nt expression cassette containing an SV40 early promoter and SV40 poly A site for expression in chicken cells (Figure 2). Currently, we are in the process of generating the modified pBeloBac 11.

In terms of goal #2, we have obtained pock homogenates of the Townsend (North Carolina) strain of ILTV. This has been adapted to replication in LHM (leghorn male hepatoma) cells and DNAs have been prepared from 3 different fractions from the infected cells (supernatant medium, cell lysates and total infected cells). We are currently evaluating these DNAs for the ability to generate infectious virus. Should these not prove to be infectious, or of extremely low titer, we will repeat the DNA purification using a nucleocapsid isolation method recently optimized for MDV nucleocapsid and DNA purification (Dr. Stephen Spatz, Journal of Virological Methods, in press).

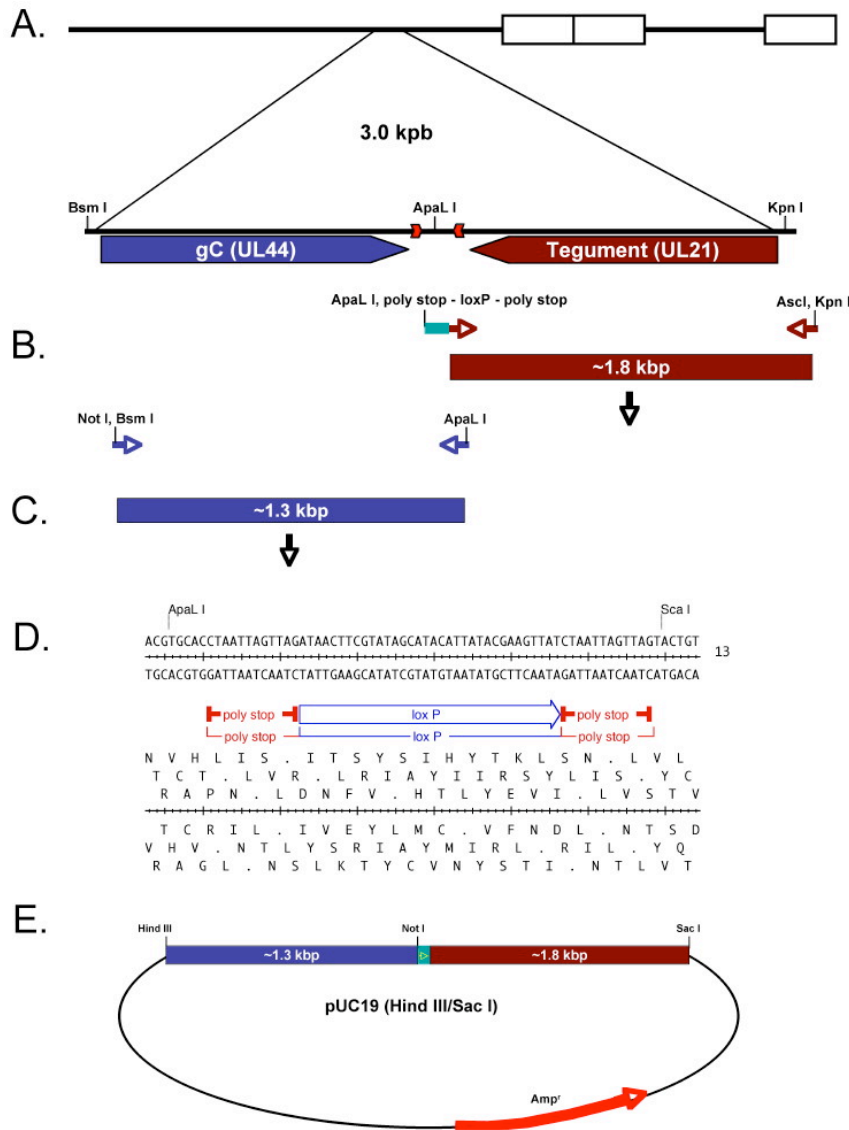


Figure 1. Diagram of ILTV Transfer Vector Construction. Panel A. depicts the ILTV genome with an amplified insert of the region for BAC insertion. This region contains an intergenic segment between UL44 (gC) and UL21 (tegument) genes, each of which has a downstream transcriptional terminator (AAUAAA, red arrowheads). Panels B and C depict the individual PCR products and additional sequences introduced with extended primers, and the product sizes (in kbp). Panel D shows the poly stop – loxP – poly stop sequences introduced in this intergenic region. Below the colored bars depicting the poly stop (red) and loxP (blue) sequences are the three forward and three reverse open reading frames (single letter code). In these frames, periods “.” denote stop codons. Panel E. depicts the vector for BAC introduction that is currently in construction. This vector is pUC19-derived and will be used for insertion of the BAC sequences at the single loxP site (green box with yellow arrow).

Statement Regarding Project Status and Future Endeavors

Despite the termination of the funding period (June 30, 2008), this project is being continued. The original project period was somewhat unrealistic, in terms of the ability to generate infectious clones within the six months of funding, and despite some personnel issues, we have made steady progress on this project.

We plan on the continuation of this project, as the derivation of ILTV infectious clones, the development of a more rapid method for doing so, and the products that these clones promise to yield, is of high priority for our laboratory, our department and our constituency in the state of Delaware.

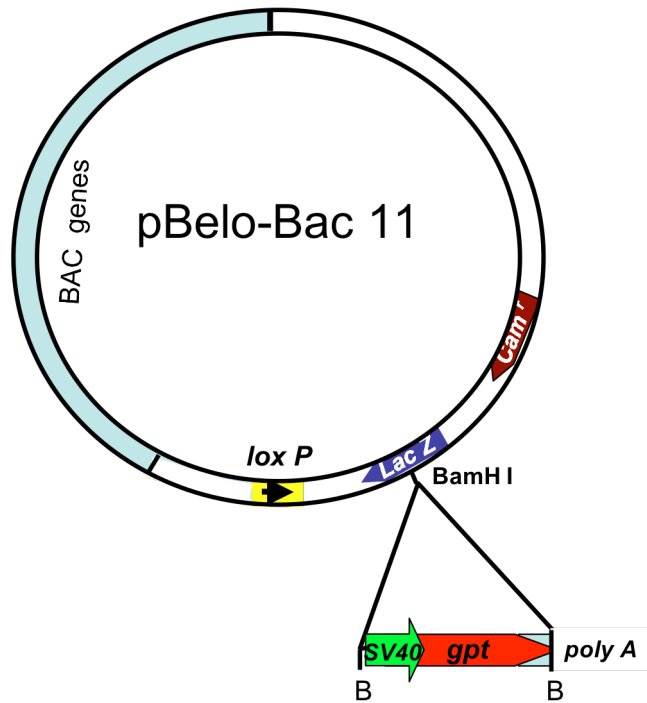


Figure 2. Diagram of the modified BAC vector, pBelo-BAC 11gpt. At left is a schematic diagram of pBelo-BAC 11. This vector contains the replication proteins and origin of replication to maintain foreign sequences at a low copy number as a bacterial artificial chromosome within *E. coli* (light blue region). pBelo-BAC 11 contains a single loxP recombination site (yellow box with black arrow), a lacZ alpha peptide-coding region for blue/white selection (blue arrow) and a chloramphenicol resistance gene (Cam^r, maroon arrow). A gpt cassette has been PCR amplified from vector pVEC-12, having the SV40 early promoter (green arrow), the gpt coding sequence (in red) and a modified SV40 poly adenylation signal (light blue box). For rapid cloning, this cassette was generated with *Bam*H I sites being introduced at either end (B). This cassette has been cloned and sequenced and will now be inserted at the unique BamH I site in pBelo-BAC 11.