Example Category I Summary Information Format
Feline Immunodeficiency Virus Vaccine, Inactivated Subunit

I. Introduction

A. Objective

1. Identify where the Regulated Biological Agent was constructed and where the product will be made, tested and manufactured. Address the available level of containment.

Feline Immunodeficiency Virus Vaccine, Inactivated Subunit, Product Code 1234.RO was constructed from the agents listed below at VAXMAX Corporate Research and Development, Cloneready, MT. The final vaccine will be produced at VAXMAX Manufacturing, Cloneready, MT, U.S. Veterinary License No. 000. VAXMAX has level 2 and 3 animal containment facilities available for testing of live vectors.

2. Provide a brief (one sentence) description of the Regulated Biological Agent.

The Regulated Biological Agent (RBA), designed SPVtk-FIVenv, will contain a swine poxvirus (SPV) backbone or vector component and will express the envelope gene of feline immunodeficiency virus (FIVenv). The SPVtk-FIVenv cell harvest will be inactivated with beta propiolactone and administered subcutaneously to cats.

B. Proposal

1. What is the intended use of the product?

a. Species: *Cats, age 8 weeks or older*
b. Proposed claim: *Feline Immunodeficiency Virus Vaccine, Inactivated Subunit is indicated for use in healthy cats as an aid in the prevention of Feline Immunodeficiency Disease caused by Feline Immunodeficiency Virus.*
c. Geographic area: *United States (all states)*
d. Route of administration: *Subcutaneous*
e. Brief description of the expected safety profile: *FIVenv will disrupt the thymidine kinase gene of SPV, attenuating the wild-type virulence phenotype. The SPVtk-FIVenv is administered as a killed subunit; it does not show any site reactions nor can it shed.*

II. Description of the Regulated Biological Agent Construction

A. The Backbone Biological Agent

1. What organism was used for the Backbone Biological Agent? Are there any known virulence features associated with the Backbone Biological Agent? What happens in the target species?

Swine poxvirus prototype I is a double-stranded linear DNA virus in the Poxviridae Family (Figure 1). The parent strain causes skin lesions in young pigs 3-4 weeks after exposure. The lesions are mild and duration of viral shedding is 3-4 days. SPV infection is not considered productive (viable) in felines.

a. What is the previous safe use of the Backbone Biological Agent?

Previous use of SPV in cats has not been described. SPV is a Biosafety Level 2 by recommendation of CDC/NIH.

2. Physical characteristics of the Backbone Agent

a. Provide a flow diagram or explanation of the process of how the Backbone Biological Agent was constructed

[Figure 1] [Figure 2] [Figure 3] [Figure 4]

(i) Describe the proposed site for Donor DNA insertion.

See figures.

(ii) Do the flanking regions of the proposed insertion site in the Backbone Biological Agent have any known regulatory elements that could moderate the expression of the inserted donor DNA?

The flanking regions of the insertion site will be the 5' half and the 3' half of the tk gene of SPV. The tk gene has a typical eukaryotic promoter and poly A addition sites.

(iii) Identify unique restriction endonucleases (not more than five) that will give identifiable digestion patterns useful for characterizing the final Backbone DNA.

The entire tk gene and flanking DNA can be isolated on a 650-bp Mst I and Kpn I digest of the SPV genome.

B. Donor Biological Agents and Donor DNA or Genes

1. What are the Donor Biological Agents used as the source of each Donor DNA sequence inserted into the Backbone Biological Agent?

The description of each donor biological agent is shown in Figure 3 and Figure 4. The promoter, poly A addition site, and the multiple cloning sites are derived from the Stratagene® Vector pCMVScript™. A synthetic 62-base pair poly A addition site (Young, et. al. 1982. Gene 52: 251-257, attached) made by oligo DNA synthesis was added 5' to the CMV promoter to prevent read-through of the SPV tk gene into the inserted DNA. The inserted Donor DNA sequence is a 1500-bp PstI-PstI fragment taken from a double-stranded DNA (cDNA) form of the FIV genome (see Figure 2).

a. Has there been safe use of the Donor Sequences or Donor Genes, as well as Safe use of the Donor Biological Agent?

The FIV isolate came from an indoor/outdoor cat (5 years of age); it is a subtype A. A clarified preparation of the virus is available. There is no known previous use of this isolate. Previous use of envelope genes from several FIV isolates has been described (Pederson, et. al. 1986).

b. Are there specific parts of the Donor Gene(s) or Sequences that were used for insertion? Show pertinent sequences or restriction endonuclease sites.

The donor gene is the envelope (env) gene of FIV. The gene is isolated from double-stranded DNA (cDNA) by converting the single-stranded RNA genome of FIV to a ds cDNA using an in vitro reverse transcriptase assay (See Figure 2 and Figure 4). Restriction enzyme Pst I digests the env gene cDNA at two sites that are 21 base pairs downstream of the start codon and 33 base pairs upstream

from the 3' poly A addition site, respectively, producing a 1500-base pair segment that includes the major env epitopes of FIV (see Figure 1).

C. Construction and Characterization of the Regulated Biological Agent

1. Provide a flow diagram on the construction of the Regulated Biological Agent.

   a. Final Backbone Biological Agent *(Figure 1)*

   b. Donor DNA or gene *(Figure 2)*

   c. All shuttle vectors *(Figure 3 and Figure 4)*

   d. Host cell lines used: *E. coli DH5a was used to transform and amplify the shuttle vectors. Feline kidney cells were used as the host cell line for recombination of transfected vector DNA containing the FIVenv gene inserted into the tk locus, and infection with wild-type SPV prototype I (Figure 4).*

   e. Selection techniques and methods used to construct the final Regulated Biological Agent: *Virus successfully growing in media supplemented with 5-bromodeosuridine was considered to be tk negative. Plaques were picked and amplified three times to isolate and expand clones of SPVtk-FIVenv.*

2. Describe the laboratory methods or criteria used to evaluate the Regulated Biological Agent.

   Gene insertion was characterized by genetic detection described in II.C.3.a-c. The FIV env product was determined by Western blot of CrFK cells expressing env gene product of 58 Kd, using a monospecific antibody to gp70 protein of FIV.

3. Physical characterization of the Regulated Biological Agent

   a. Characterize the physical map, using unique Donor DNA and Backbone Biological Agent restriction endonuclease sites, and describe resulting restriction fragments and digestion patterns.

      Restriction digest size and patterns are provided in Figure 5.

b. Devise a PCR or restriction endonuclease test based on the Backbone Biological Agent sequence and the Donor Sequence that will identify and characterize the Donor DNA/Backbone Biological Agent construct.

PCR product of 2.54 kb, using a single forward and reverse primer, defines the insert and tk-specific sequences.

c. What will be the criteria for stability and purity of the Regulated Biological Agent Master Seed n and n+5?

Restriction digest and PCR pattern defined in Figure 5 will be used to test MSV n and n+5.

d. Provide the genetic sequence in electronic format for any new or altered genetic sequences, including insertion and flanking sequences.

(electronic file attached)

4. What is the recommended CDC/NIH biosafety level for the Regulated Biological Agent?

The Regulated Biological Agent is attenuated for replication, as demonstrated by consistently lower titers in MA105 cells (Log_{10} GMT = 6.5 $TCID_{50}$/mL) when compared to the parental strain (Log_{10} GMT = 7.5 $TCID_{50}$/mL). See detailed report in Appendix I. Because the FIV env does not provide any new virulence factors to the SPV backbone and because the SPVtk-FIVenv is attenuated compared to the parent virus, the recommended biosafety level will be the same as the parent virus (BL2).

5. Provide a short summary or description of genetic motifs that may have resulted as a consequence of the genetic recombination (II.C.3.d). Are there any known motifs that may promote homologous recombination, DNA insertion, or gene expression of existing or new open reading frames?

These include the bovine growth hormone, SV40 poly A addition site, and the CMV promoter. All have been used previously for production of safe mammalian expression vectors. The bacterial plasmid components are derived from a well defined and utilized pCMV Script, which is a derivative of pUC-based vectors distributed by Stratagene. Vectors, such as pUC, and host strains, such as E. coli DH5a, are considered to be Biosafety Level 1.

Figure 1. Swinepox Virus Backbone Biological Agent

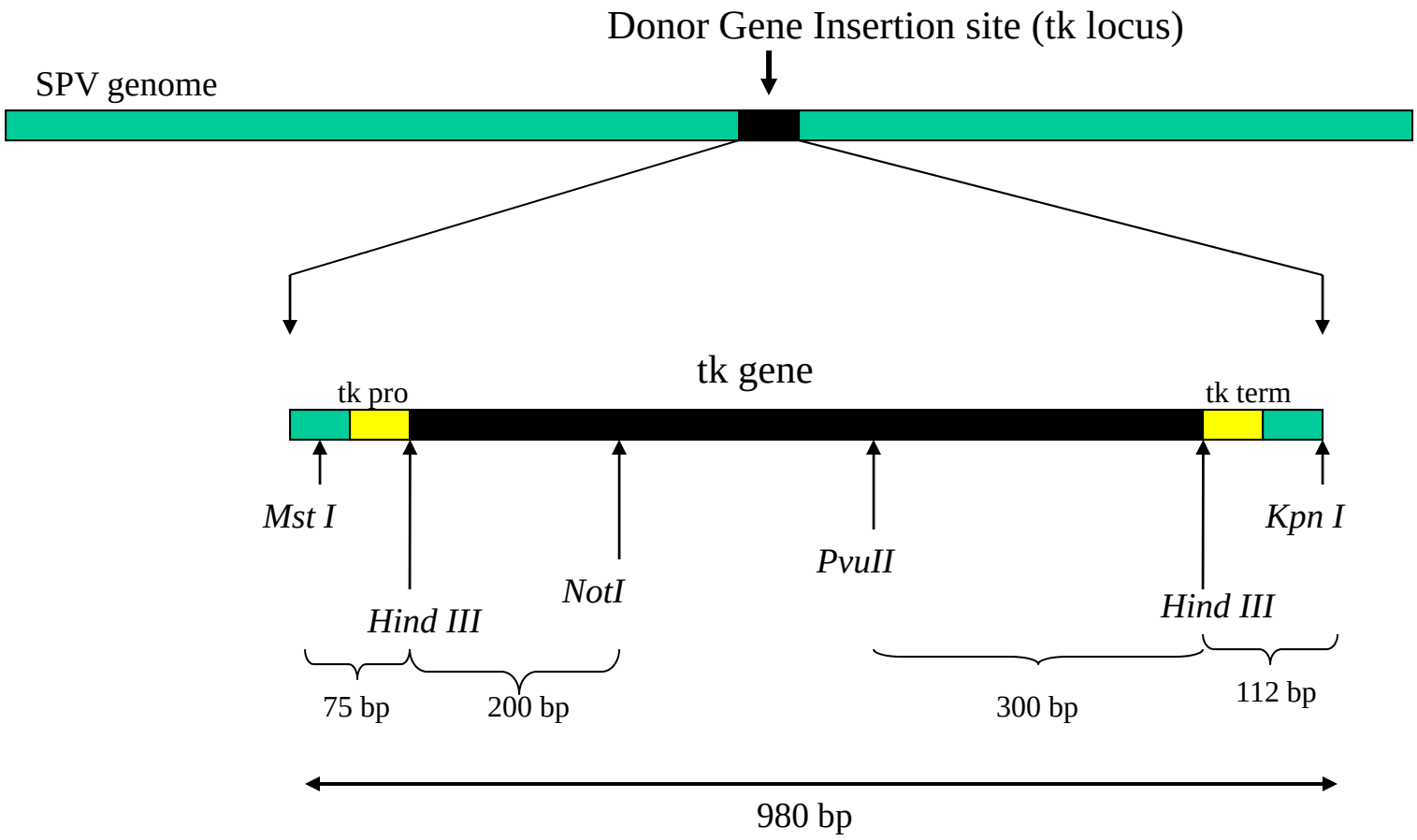


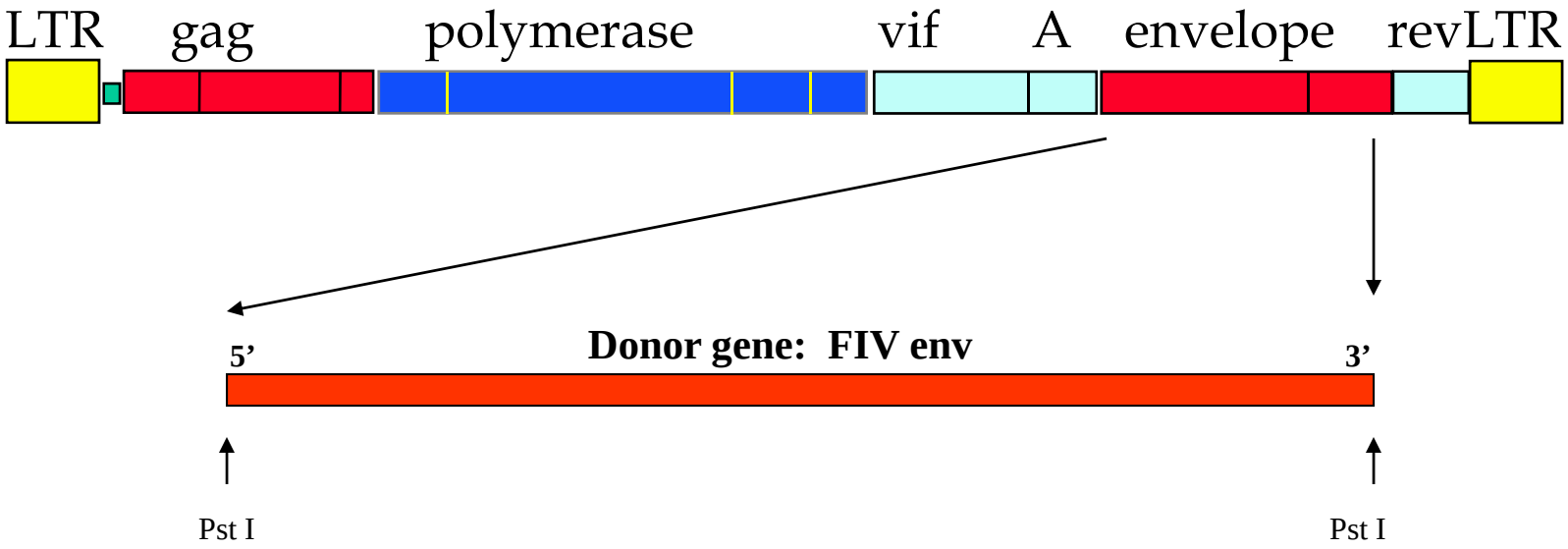
Figure 2. Donor Biological Agent

Virus gene regulation

Genes for structural proteins

Genes for enzymes

Genes for regulatory proteins



Pst I restriction enzyme digest removes full length env gene minus 24 nucleotides from 5’ N-terminus to the poly A addition site 3’ C-terminus (1500 bp)

Figure 3. Production of Expression Vector pCMVex

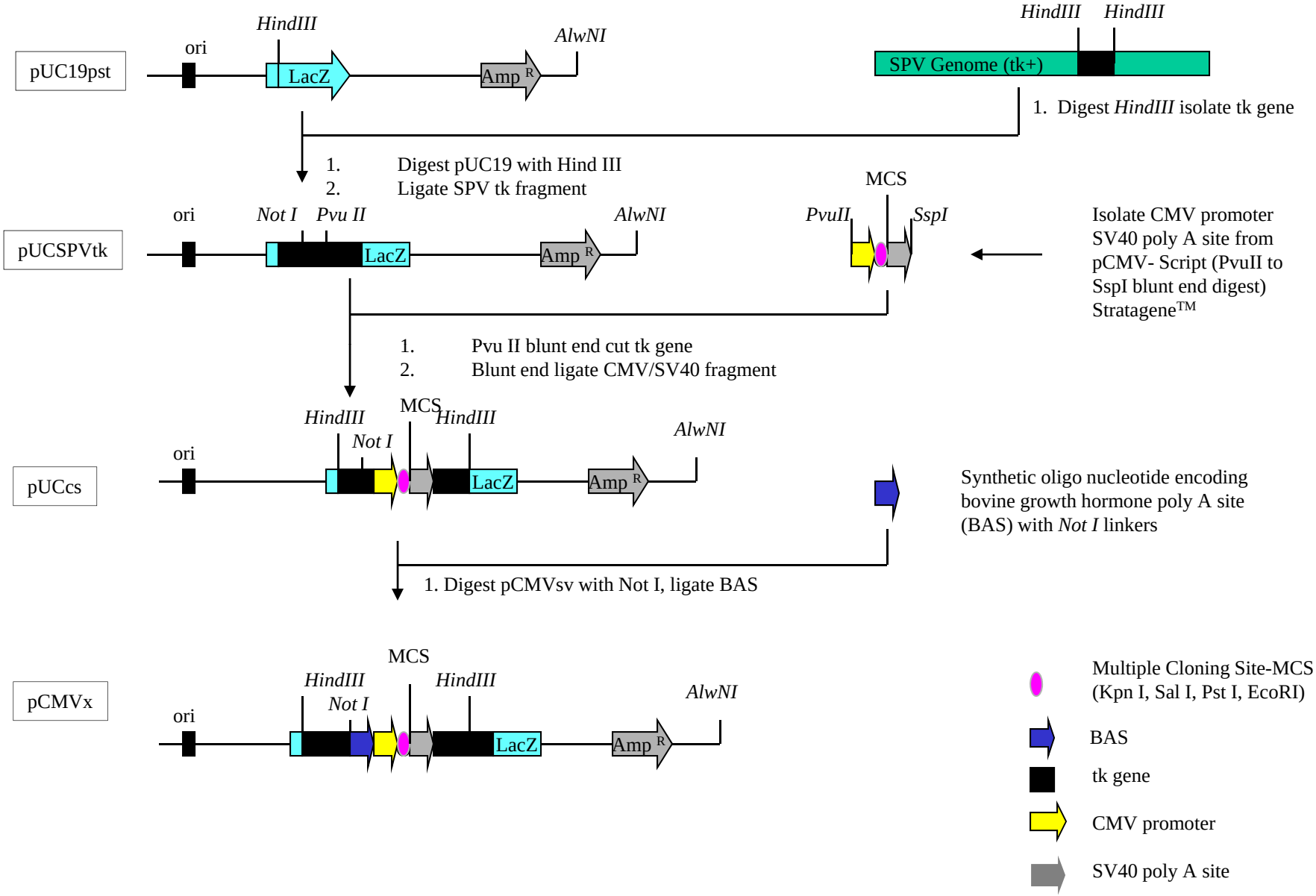
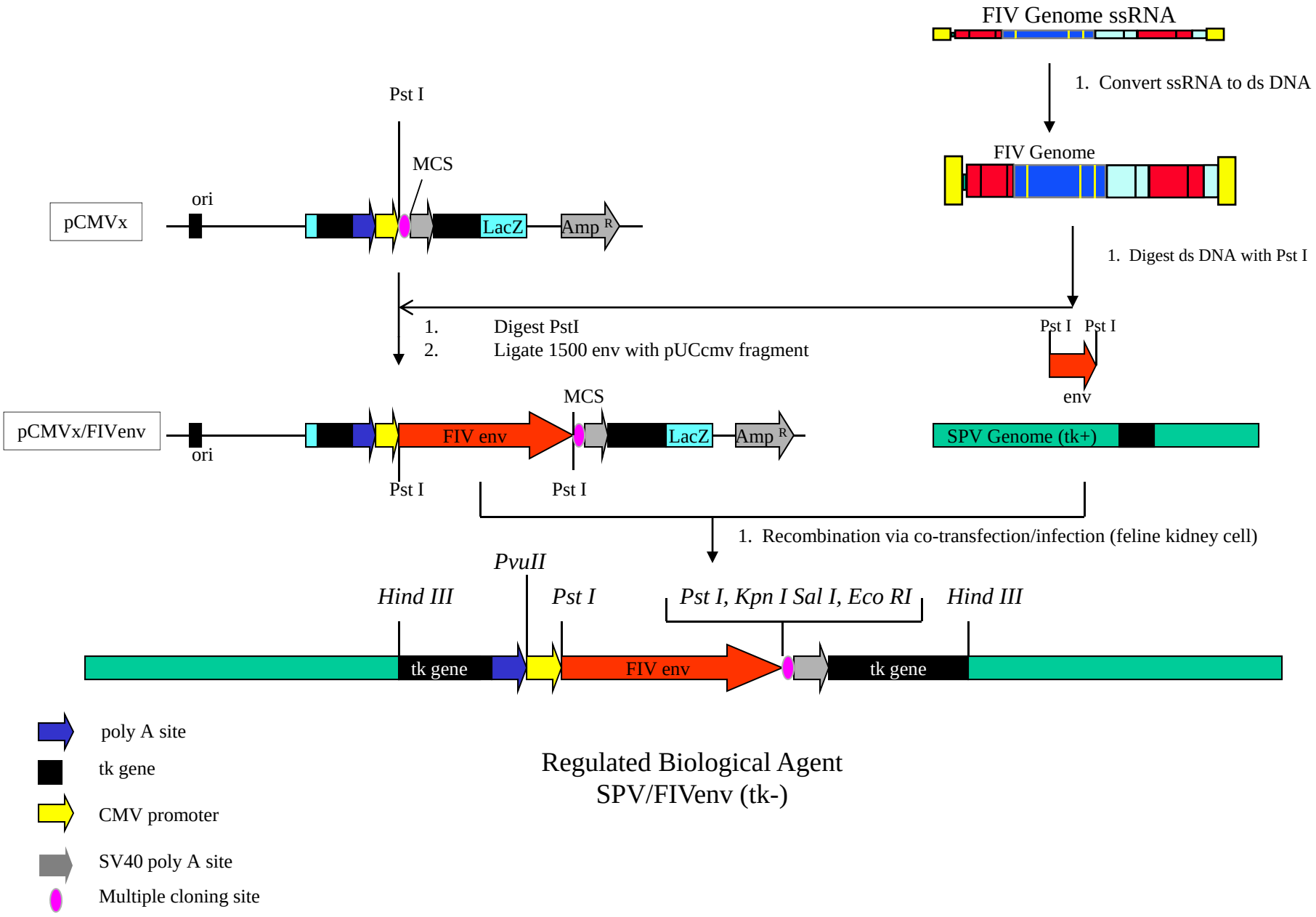


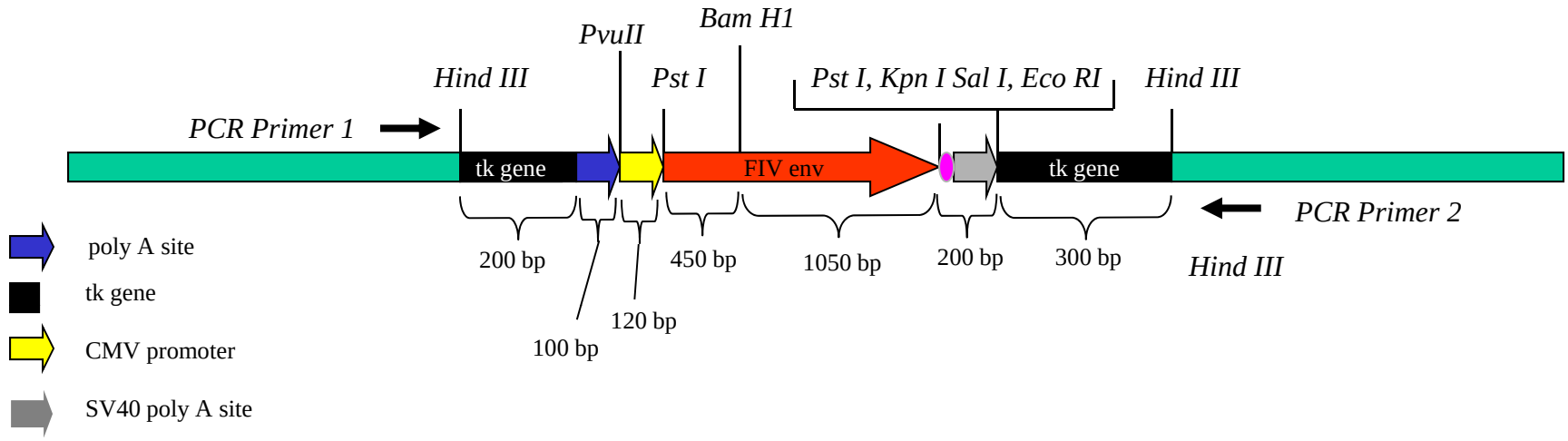
Figure 4. Production of the Regulated Biological Agent SPV/FIVenv(tk-)



Regulated Biological Agent
SPV/FIVenv (tk-)

Figure 5. Regulated Biological AgentSPV/FIVenv (tk-)

Construct Characterization based on
PCR and Restriction endonuclease digestion



PCR Product and Restriction Endonuclease Characterization

Restriction Enzymes	Digestion Product Sizes (bp) and Generated Fragments			
Hind 111	2420			
Hind 111 Bam H1	1550	870		
Hind 111 Bam H1 Pst 1	1250	450	420	300
Hind 111 Bam H1 Pvu 11	1550	570	300	
PCR Product	2450 (Primers 15 bases each)			