Example Category III Summary Information Format
Feline Immunodeficiency Virus Vaccine, Live Vector

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, Live Vector, Product Code 1236.R0, 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), designated SPVtk-FIVenv, will contain a swine poxvirus (SPV) backbone or vector component and will express the envelope gene of feline immunodeficiency virus (FIVenv).

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, Live Vector, 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, attenuating the wild-type virulence phenotype of the swine poxvirus vector. Administration of the SPVtk-FIVenv will cause a transient viremia in the cat but no shedding in feces, saliva, or other body fluids. No prolonged itching or irritation is expected at the site of inoculation.*

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. The parent strain causes skin lesions in young pigs 3-4 weeks after exposure. The lesions are mild, and virus is shed for 3-4 days. SPV infection is not considered productive (viable) in felines.

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

(i) If available, provide history of previous safe use, using published literature or internal documents. Include the recommended CDC/NIH biosafety level for use of the Backbone Biological Agent.

Previous use of SPV in cats has not been described. SPV is a Biosafety Level 2 pathogen 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 the 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.

See Figure 5. The entire tk gene and flanking DNA can be isolated on a 980-bp Mst I and Kpn I digest of the SPV genome. (See Figure 1.)

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?

(i) Provide relevant references for safe use.

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 was 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.

   Include the following:

   a. Final Backbone Biological Agent: *Figure 1*
   b. Donor DNA or gene: *Figure 2*
   c. All shuttle vectors: *Figure 3* *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 HPRT-depleted media were 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 detected 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?

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 and 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 pathogens.

III. Biological Properties or Virulence for the Regulated Biological Agent used for Master Seed

A. What are the known phenotypic characteristics or effects of the Regulated Biological Agent identified as Master Seed?

1. The RBA lacks thymidine kinase activity, as demonstrated by replication in the presence of the thymidine analog, 5-bromodeoxyuridine. (See Appendix I for a detailed report.)

2. The RBA 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 Appendix II for a detailed report.

3. The RBA expresses the envelope gene of FIV, as demonstrated by blue plaque assay. When virus dilutions are plated onto MA105 cells and overlayed with agarose, the plaques that develop can be stained in situ with a monoclonal antibody (conjugated to horseradish peroxidase) specific for the FIV envelope protein. Plaques that bind the conjugated antibody turn blue when incubated with a peroxidase substrate. (See Appendix III for a detailed report.) The envelope protein is expressed as a full-length protein, as shown by Western blot analysis of infected culture lysates. The monoclonal antibody against FIV envelope protein stains a 138kd protein, which is the expected expressed protein size for this construct. (Seep Appendix IV for a detailed report.)

B. What are the virulence characteristics of the Regulated Biological Agent used for the Master Seed?

1. The RBA is replication defective in the following feline cells: feline kidney cell line (CRFK), feline lung cell line (AK-D), fibrosarcoma cell line (FC65.T), and primary feline fibroblasts. (See Appendix V for a detailed report.) Therefore, it is not expected to be virulent in cats.

2. The RBA does not contain any genetic elements or toxin genes that are known to be inherently virulent.

C. Is the Regulated Biological Agent used for the Master Seed virulent for target animals, non-target animals?

1. The master seed virus was tested for virulence in cats by subcutaneous administration of virus (Log_{10} titer = 8.5 $TCID_{50}$) ten 8-

week-old kittens. This dose represents ten times the expected field dose for the vaccine. Kittens were monitored for clinical signs, including, but not limited to, skin lesions, fever, injection site reactions, depression, lethargy, inappetance, vomiting, diarrhea, nasal discharge, and conjunctivitis. Mild injection site reactions (palpable soft tissue inflammation) were found for 1-2 days; however, no other clinical signs were observed in any kittens over the 21-day observation period. (See Appendix VI for a detailed report.) Taken together with the replication defective nature of this virus in feline cells, these data indicate that the RBA is not virulent in cats.

2. The master seed was tested for virulence in pigs and compared to the parental strain. Five 1-week-old pigs were inoculated intradermally with the RBA and five inoculated with the parental strain. Five doses of 0.1 mL were administered to each pig (Log_{10} titer = 8.0 $TCID_{50}$/pig). Pigs were monitored for 21 days for clinical signs typical of swine pox. Pigs inoculated with the parental strain developed fever and skin lesions typical of swinepox. There was an average of 15 lesions per pig that lasted for an average of 6 days. The RBA did not cause skin lesions or any other clinical signs in inoculated pigs. (See Appendix VII for a detailed report.) Therefore, the RBA appears to be attenuated for the potential to cause clinical disease in pigs.

3. The master seed was tested for safety in mice according to 9 CFR 113.33; no adverse events were observed. (See Appendix VIII for a detailed report.)

4. The master seed was tested for safety in guinea pigs according to 9 CFR 113.38; no adverse events were observed. (See Appendix IX for a detailed report.)

D. Do the Donor DNA sequences enhance the virulence or the ability of the Master Seed to survive in the target animals, non-target animals?

1. What is the tissue tropism of the Regulated Biological Agent in target and non-target animals?

a. Ten 1-week old pigs were inoculated intradermally with the parent swine poxvirus or the RBA (Log_{10} titer = 8.0 $TCID_{50}$). Two pigs were necropsied on each of the following days: 3, 5, 7, 10, and 14. At necropsy, tissues were obtained from skin at the injection site, lymph nodes draining the injection site, thymus, spleen, liver, kidneys, heart, tonsils, and intestines. Tissues were homogenized and tested for the presence of swine poxvirus or RBA, using a PCR assay. The parental strain was detected in tissues at the injection site (days 3, 5, 7, and 10) and the draining

lymph nodes (day 10), but not from any other tissue examined. The RBA was detected in the tissue from the injection site (day 3), but not from any other tissue examined. (See Appendix I for a detailed report.) These data indicate that the RBA has a reduced tissue distribution when compared to the parental strain and persists for a shorter time in pigs.

b. Ten 8-week-old kittens were inoculated subcutaneously with RBA (Log_{10} titer = 8.5 $TCID_{50}$). Two kittens were necropsied on each of the following days: 3, 5, 7, 10, and 14. At necropsy, tissues were obtained from skin at the injection site, lymph nodes draining the injection site, thymus, spleen, liver, kidneys, heart, tonsils, and intestines. Tissues were homogenized and tested for the presence of swine poxvirus or RBA, using a PCR assay. The RBA was detected in the tissue from the injection site (day 3), but not from any other tissue examined. (See Appendix XI for a detailed report.) These data indicate that the RBA is restricted to the injection site tissue in vaccinated cats.

2. Provide direct scientific evidence, including any relevant scientific publications, if available.

The restricted host range of swine poxvirus has been reported by others (Barcena J and Blasco R. Recombinant swine poxvirus expressing beta-galactosidase: investigation of viral host range and gene expression levels in cell culture. Virology 243: 396-409, 1998).

E. Discuss the potential for horizontal gene transfer or recombination of the Regulated Biological Agent.

1. Is there any reason to believe that the potential for horizontal gene transfer or recombination is different in target and non-target animals?

a. Include contributions from the Backbone Biological Agent and Donor DNA Sequence in the discussion of the horizontal gene transfer and recombination potential.

The potential for the RBA to recombine with other viruses was analyzed under laboratory conditions. Crandell feline kidney cells were co-infected with the RBA and feline immunodeficiency virus. Progeny viruses were analyzed by PCR for signs of potential virus recombination. This analysis included the potential for the envelope gene from the wild-type FIV to cross into the RBA and the potential

for the RBA envelope gene to cross into the FIV virus. No evidence of cross-over was detected. (See Appendix XII for a detailed report.)

b. Reference any relevant scientific publications.

A comprehensive literature search was performed to find published evidence of horizontal gene transfer between swine poxvirus and other viruses. No publications were found. (See Appendix XIII for a copy of the literature search results.)

F. Describe the shed/spread capabilities of the Regulated Biological Agent.

1. Include shed/spread potential in non-target animal species.

a. Kittens inoculated with the master seed RBA were commingled with two uninoculated kittens during the 21-day observation period for the master seed safety test (reported in Section III.C.1). The sentinel kittens were bled at 21 and 35 days after exposure to vaccinated kittens, and their sera were tested for antibody against swine poxvirus. All of the vaccinated kittens developed antibodies to SPV, whereas both of the sentinel kittens remained seronegative. (See Appendix XIV for a detailed report.) These data, coupled to the replication defective nature of the RBA in feline cells, provides strong assurance that there is little likelihood of shed and spread of the RBA in the cat population.

b. Pigs inoculated with the master seed RBA were allowed to nurse the same sow as two uninoculated piglets during the 21-day observation period for the master seed safety test (reported in Section III.C.2). The sentinel pigs were bled at 21 and 35 days after exposure to vaccinated pigs, and their sera were tested for antibody against swine poxvirus. All of the vaccinated pigs developed antibodies to SPV, whereas both of the sentinel pigs remained seronegative. (See Appendix XV for a detailed report.) Therefore, the RBA does not appear to be capable of shed and spread in the swine population.

2. Include in the discussion any contributions to shed/spread capabilities that might be associated with particular attributes of the Backbone Biological Agent and Donor DNA sequence.

The insertion of the FIV envelope gene into the swine poxvirus vector has resulted in significant attenuation of the virus. This appears to be due to the inactivation of the thymidine kinase gene

and possible interference of the expressed FIV protein with efficient replication and assembly of progeny recombinant swine poxviruses.

3. Reference relevant scientific publications.

Swine poxvirus is reported to spread only by direct contact with infected skin lesions (Diseases of Swine, 8th Edition, Iowa State University Press, 1999, pp. 950). Therefore, the risk for shed and spread of this RBA is very low, based on the absence of open skin lesions in kittens vaccinated with an overdose of vaccine.

G. Discuss the expected environmental impact or survivability of the Regulated Biological Agent, and provide available scientific evidence.

1. Do the Donor DNA sequences or gene sequences associated with the Backbone Biological Agent enhance the ability to survive in the environment or increase resistance to therapeutic agents?

The RBA and parental strain were evaluated for survival in liquid and desiccated forms under laboratory conditions. Liquid cultures of SPV or RBA were incubated at room temperature for one year and tested for viable virus on a monthly basis. Similarly, virus spotted onto sterile filter paper strips and allowed to dry was also tested. The SPV and RBA were found to survive for 3 months in liquid form at room temperature. The SPV survived for 8 months in dried form compared to 6 months for the RBA. (See Appendix XVI for a detailed report.) These data indicate that the RBA does not have an enhanced ability to survive in the environment. This, coupled to the absence of shed and spread for the RBA, indicates a low risk for environmental contamination.

Figure 1. Swinepox Virus Backbone Biological Agent

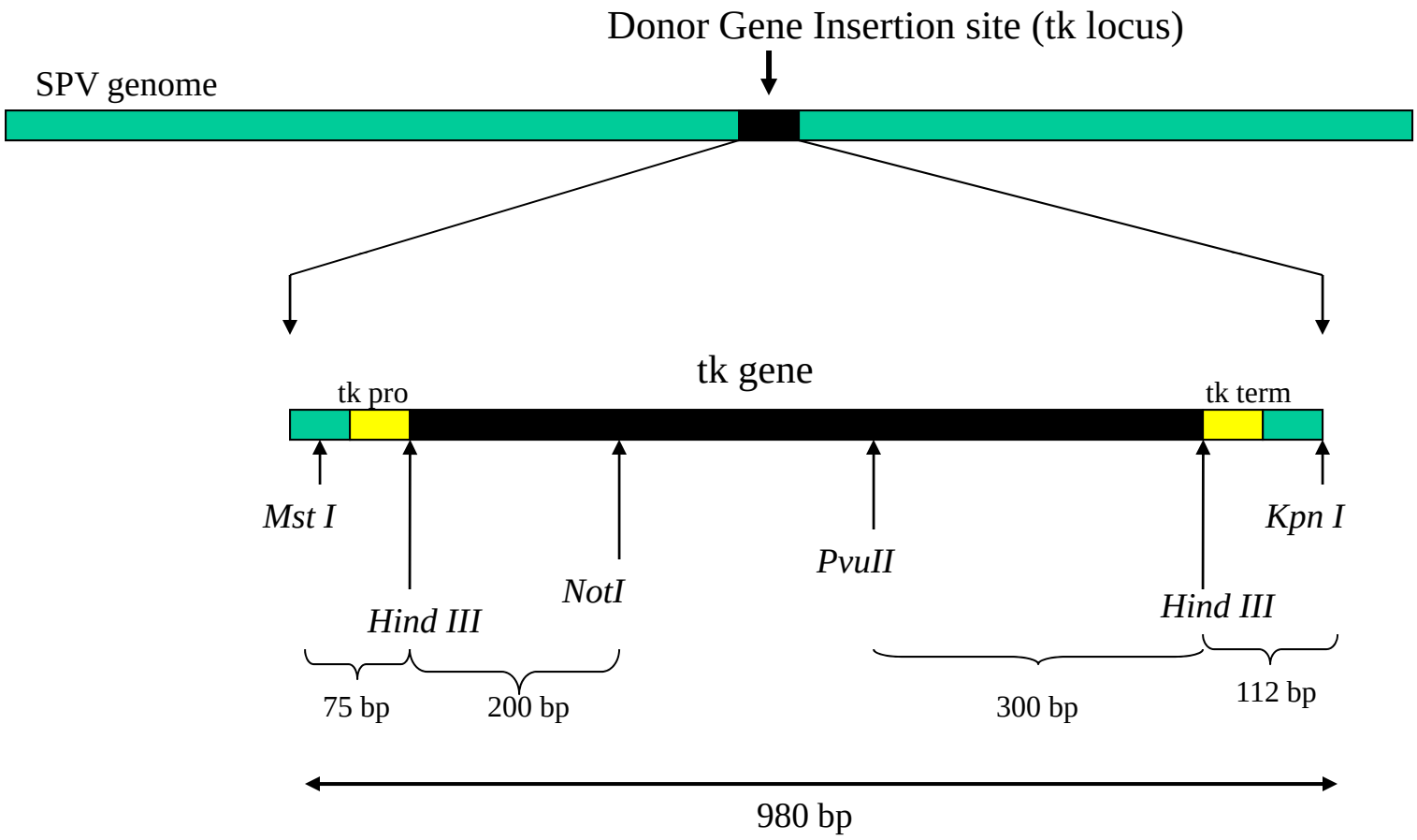


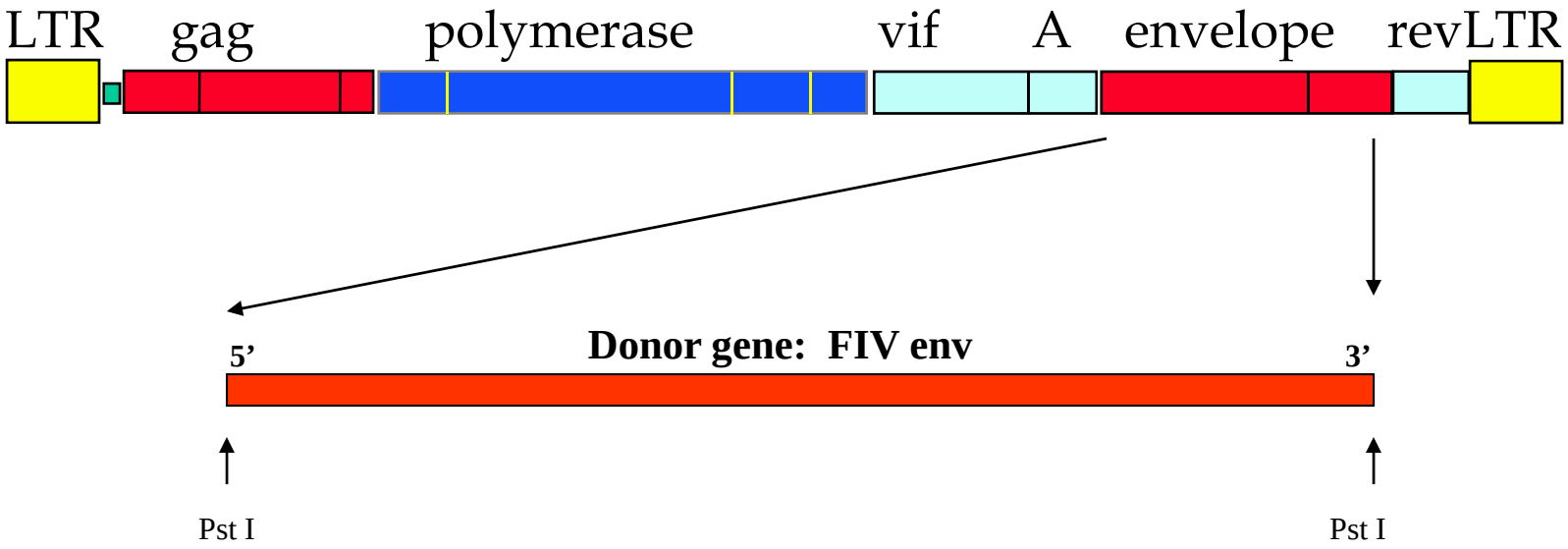
Figure 2. Donor Biological Agent

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

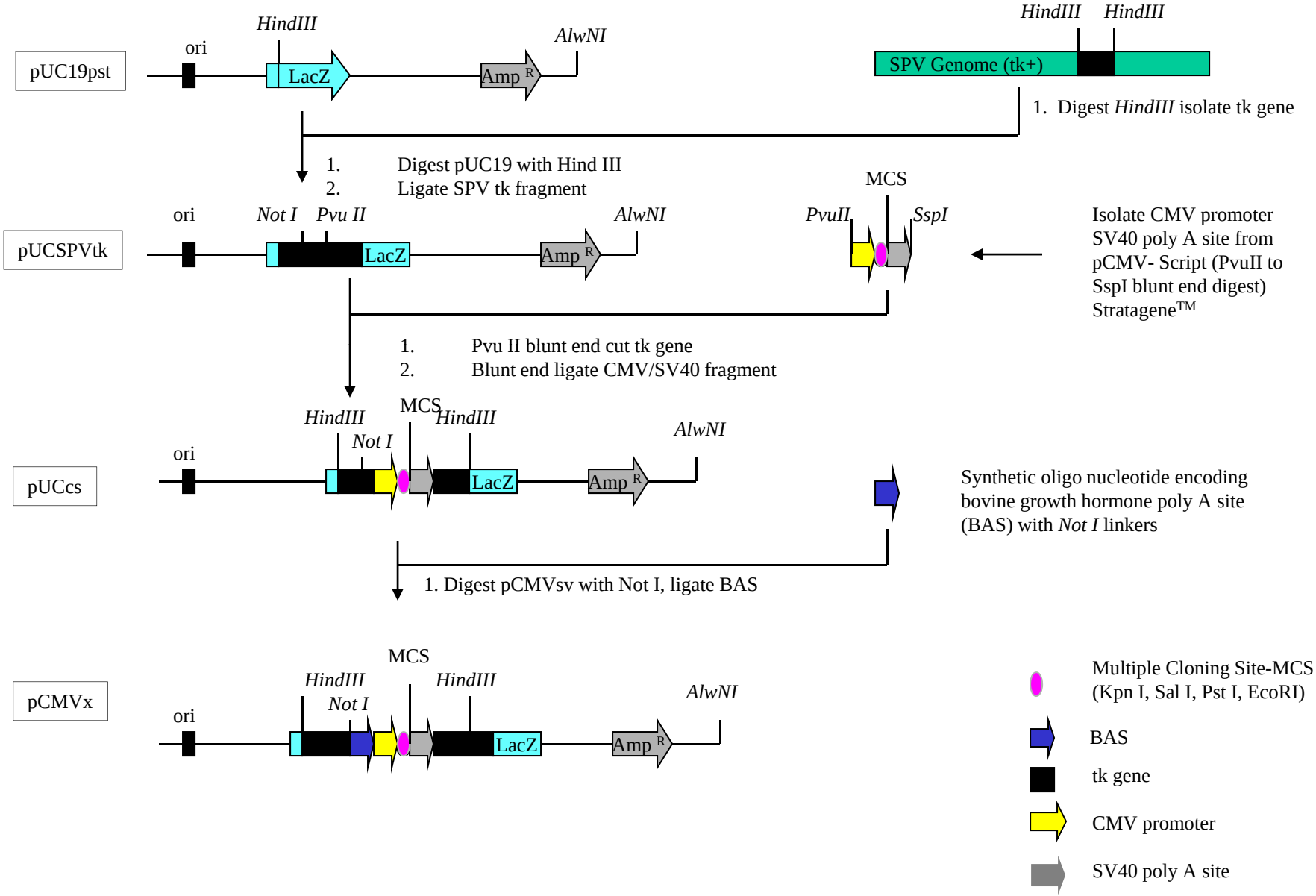
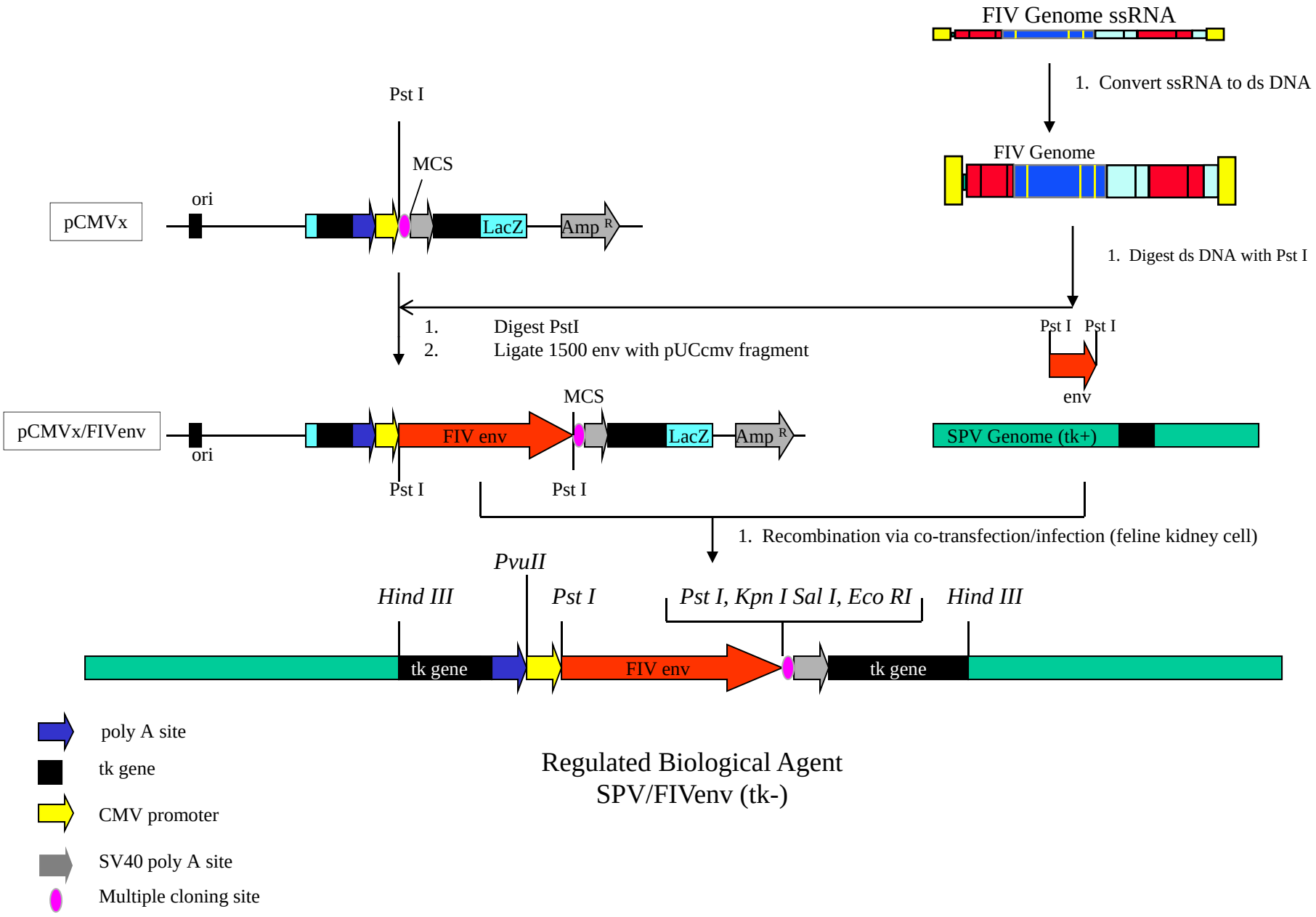


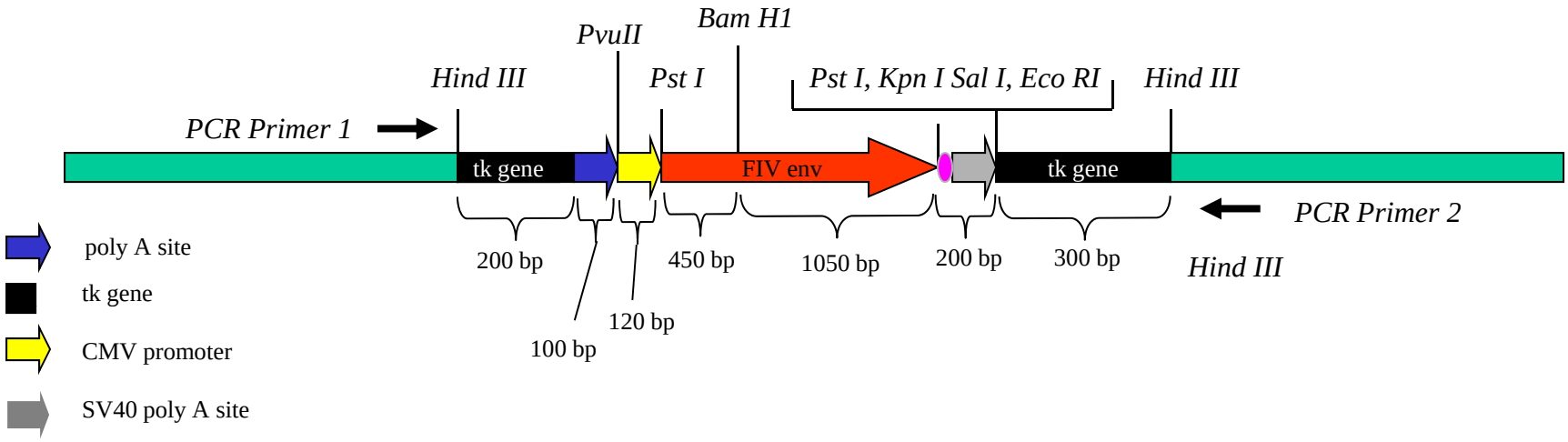
Figure 4. Production of the Regulated Biological Agent SPV/FIVenv(tk-)



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)			