Example Category II Summary Information Format
Swine Poxvirus Vaccine, Gene-Deleted (thymidine kinase negative) Live Virus

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.

The Regulated Biological Agent (swine poxvirus, thymidine kinase negative (tk-)) was constructed from the agents listed below at VAXMAX Corporate Research and Development, Cloneready, MT. The final vaccine, Product Code 1235.R0, will be produced at VAXMAX Manufacturing, Cloneready, MT, U.S. Veterinary License N. 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) will contain a swine poxvirus (SPV) backbone that is tk- and has a LacZ reporter gene, which will allow the vector to produce blue plaques in the presence of IPTG.

B. Proposal

1. What is the intended use of the product?

a. Species: *Farrowing sows and gilts*
b. Proposed claim: *Swine Poxvirus Vaccine, Live Gene-Deleted Virus, is indicated for use in healthy farrowing-age pigs as an aid in the prevention of disease associated with swine poxvirus*
c. Geographic area: *United States (all states)*
d. Route of administration: *intramuscular*
e. Brief description of the expected safety profile: *The SPV vector has a deletion in the thymidine kinase gene, attenuating the wild-type virulence phenotype. The SPVtk- will replicate transiently in the pig at the site of inoculation; no shedding in feces, saliva, or other body*

fluids is expected. 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 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.

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.

Swine poxvirus is reported to spread only by direct contact with infected skin lesions (Disease of Swine, 8th Edition, Iowa State University Press, 199, pp. 950). 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

(i) Describe the proposed site for Reporter Gene insertion.

The site for deletion is a Hind III fragment, which encodes thymidine kinase, from the Backbone Biological Agent. The deletion of the tk gene will serve as a marker to distinguish the parent virus from the gene-deleted SPVtk-. The reporter gene inserted at the site of recombination is LacZ from Escherichia coli. See Figure 1, Figure 2, and

Figure 3 for fragment size and location in the genome.

(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 Reporter Gene?

The reporter gene LacZ is driven by a CMV promoter that can be read by eukaryotic polymerases. This gene can be expressed in vitro, producing a blue plaque to help identify the SPVtk- vaccine isolate. LacZ expression does not alter the attenuated properties of SPVtk- in vitro (see reports below).

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

Figure 1, Figure 2, Figure 3, and Figure 4 identify the restriction sites of the Backbone Biological Agent DNA.

(iv) Identify reporter genes to be inserted into Backbone DNA. Show pertinent sequences or restriction endonuclease sites in the flow diagram.

Reporter gene is E. coli LacZ; see Figure 1, Figure 2, Figure 3, and Figure 4 for description of restriction endonuclease sites.

(v) Has there been previous safe use of the reporter gene? Add a citation, if appropriate.

LacZ encodes the B-galactosidase gene of E. coli. This gene has been safely used as a reporter gene in other virus vectors (see reports below).

B. 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. Heterologous reporter genes (if applicable): *Figure 3*
c. All shuttle vectors: *Figure 2*, *Figure 3*
d. Host cell lines used: *E. coli DH5a was used to transform and amplify shuttle vectors. Porcine kidney cells were used as the host cell line for recombination of transfected vector DNA and infection with wild-type SPV prototype I (Figure 2, Figure 3)*
e. Selection techniques and methods used to construct the final Regulated Biological Agent: *Figure 3 and Figure 4. The RBA lacks thymidine kinase activity, as demonstrated by replication in the presence of the thymidine analog 5-bromodeoxyuridine (5-BdU). Virus plaques isolated in the presence of 5-BdU were reisolated twice to ensure that individual virus populations were derived from a single plaque. Virus was grown in media containing IPTG, which allows the recombinant virus plaques to be blue, signifying the expression of LacZ.*

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

Gene insertion was characterized by restriction fragment analysis, as shown in Figure 3 and Figure 4. The phenotype was characterized by growth in the presence of 5 BdU, as described above.

3. Physical characterization of the Regulated Biological Agent

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

See Figure 4.

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

See Figure 4.

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

The RBA will need to be productive in the presence of 5-BdU and non-productive in the absence of 5-BdU after 5 passages from master seed. The RBA will need to retain, between n and n+5, the restriction digestion pattern described in Figure 4.

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 SPVtk- 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?

The new motifs introduced are a CMV promoter and SV40 polyA addition site that drive expression and termination of a LacZ gene. All components are derived from pBK-CMV from Stratagene.

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?

The RBA lacks thymidine kinase activity, as demonstrated by replication in the presence of the thymidine analog 5-bromodeoxyuridine. The restriction digest patter of the recombination site is described in Figure 4. (See detailed report in Appendix I.)

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 cells: porcine kidney cell line (PK), primary porcine kidney, and swine testicular cells.

Therefore, it is not expected to be virulent in pigs. (See detailed report in Appendix II.)

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 detailed report in Appendix III.)

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

1. The master seed was tested for virulence in pigs and compared to the parental strain. Five 1-week-old pigs were inoculated with the RBA and five inoculated with the parental strain. Inoculation was done by the intradermal route with five doses of 0.1 mL administered to each pig (total Log_{10} virus 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 signs. (See Appendix IV for a detailed report.) Therefore, the RBA appears to be attenuated for the potential to cause clinical disease in pigs.

2. The master seed virus was tested for virulence in cats by administration of virus (Log_{10} titer = 8.0 $TCID_{50}$) by the subcutaneous route to ten 8-week-old kittens. 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 V for a detailed report.) Taken together with the data demonstrating that the RBA is replication defective in feline cells, these data indicate that the RBA is not virulent in cats.

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

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

D. Do the Reporter Gene sequences enhance the virulence or the ability 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?

   Not applicable.

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

   Not applicable.

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 Reporter Gene 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. Porcine kidney cells were co-infected with the RBA and the parent BBA. Progeny viruses were analyzed by PCR for signs of potential virus recombination. No evidence of cross-over was detected. (See Appendix VIII 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. (A copy of the literature search results are attached as Appendix IX.)

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

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

   Swine poxvirus is normally not virulent to non-target species. To demonstrate, 3 of 6 mice littermates were inoculated with the RBA master seed and three were not inoculated. All mice were allowed to nurse on the same dam, and observations were made over a 22-day period. The three littermates inoculated with the RBA master seed did not develop any detectable titers to swine poxvirus, nor

did the three sentinel littermates. (See Appendix X for a detailed report.) Therefore, the RBA does not appear to be capable of shed and spread in a non-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 Reporter Gene sequence.

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

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 of shed and spread of this RBA is very low based on the absence of open skin lesions in pigs.

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

1. Do the Reporter Gene 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 XII for a detailed report.) These data indicate that the RBA does not have an enhanced ability to survive in the environment. This, and the data to demonstrate that the RBA is not shed or spread,

indicate a low risk for environmental contamination with this agent.

Figure 1. Swinepox Virus Backbone Biological Agent

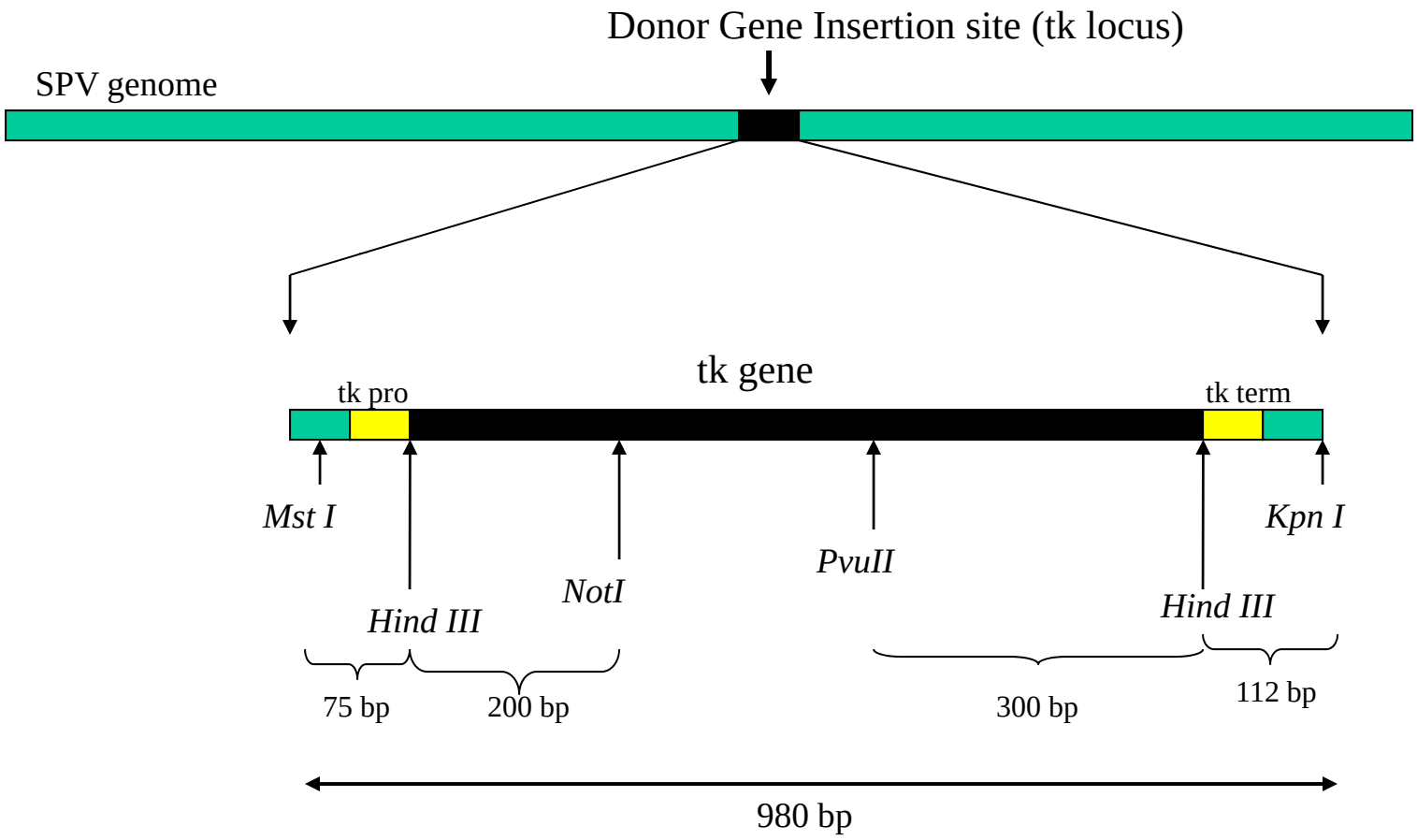


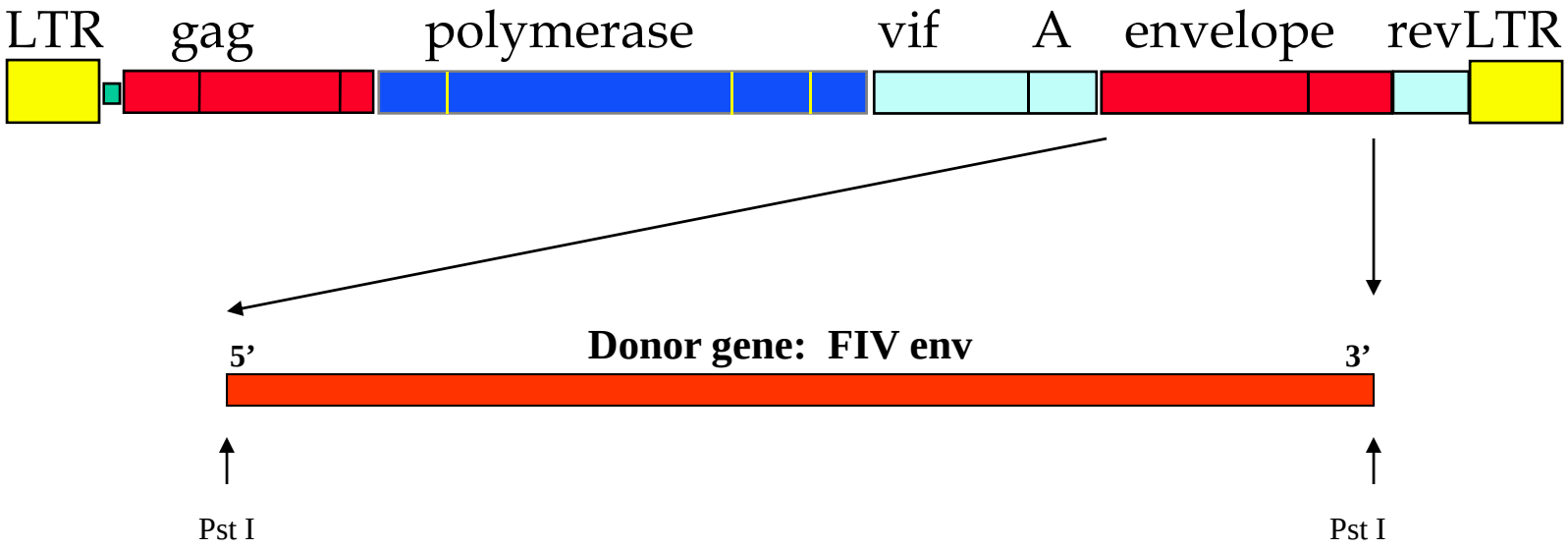
Figure 2. Donor Biological Agent

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

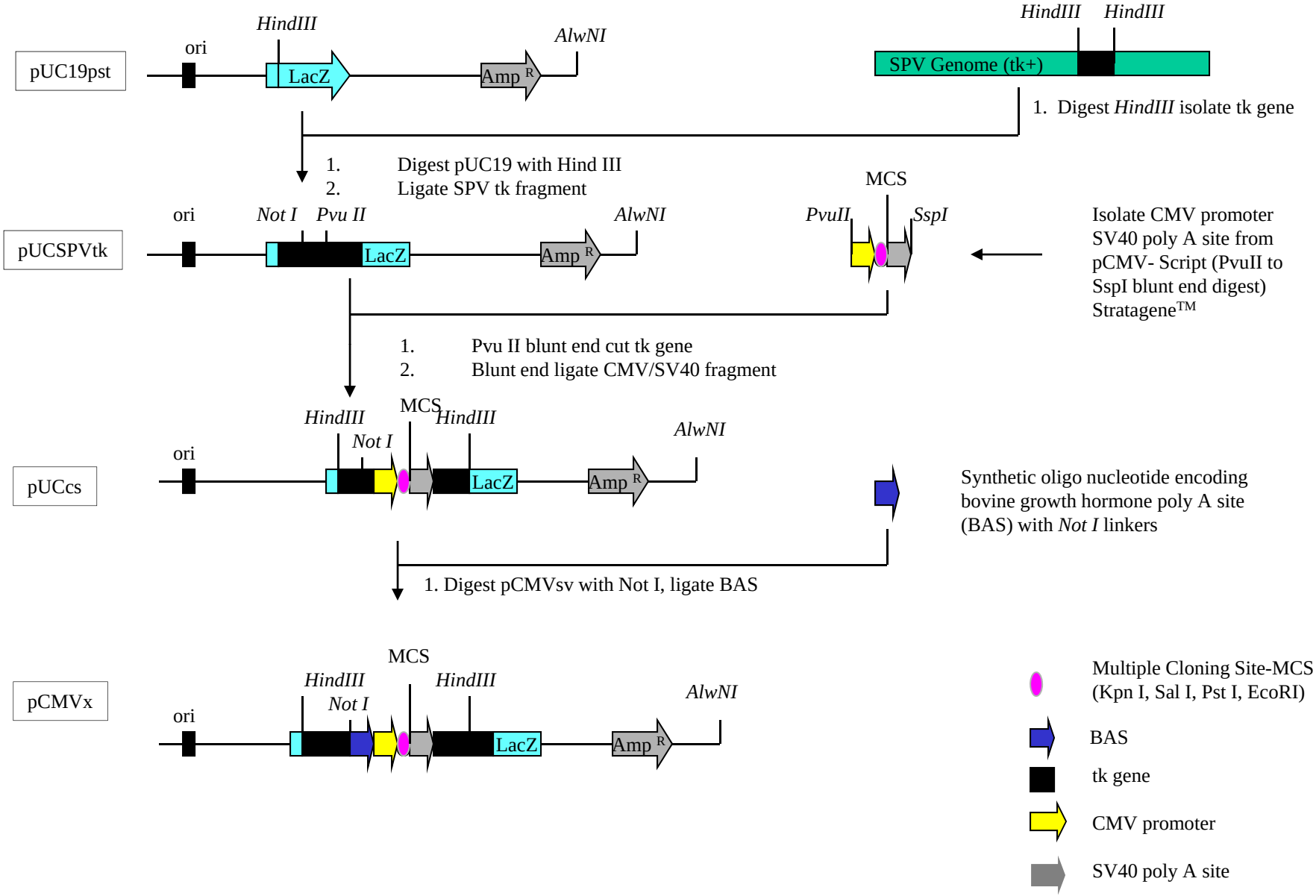
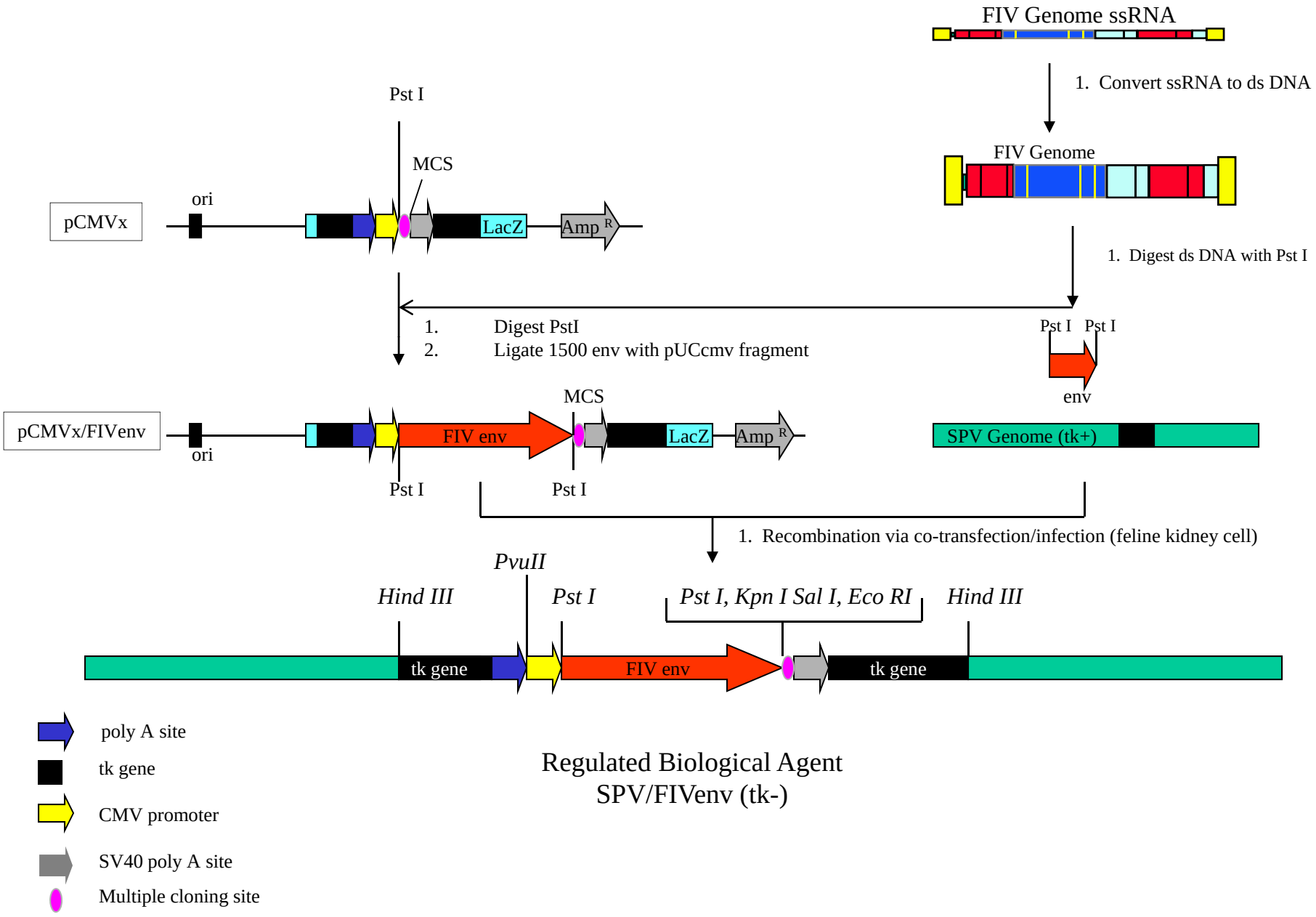


Figure 4. Production of the Regulated Biological Agent SPV/FIVenv(tk-)



Regulated Biological Agent
SPV/FIVenv (tk-)