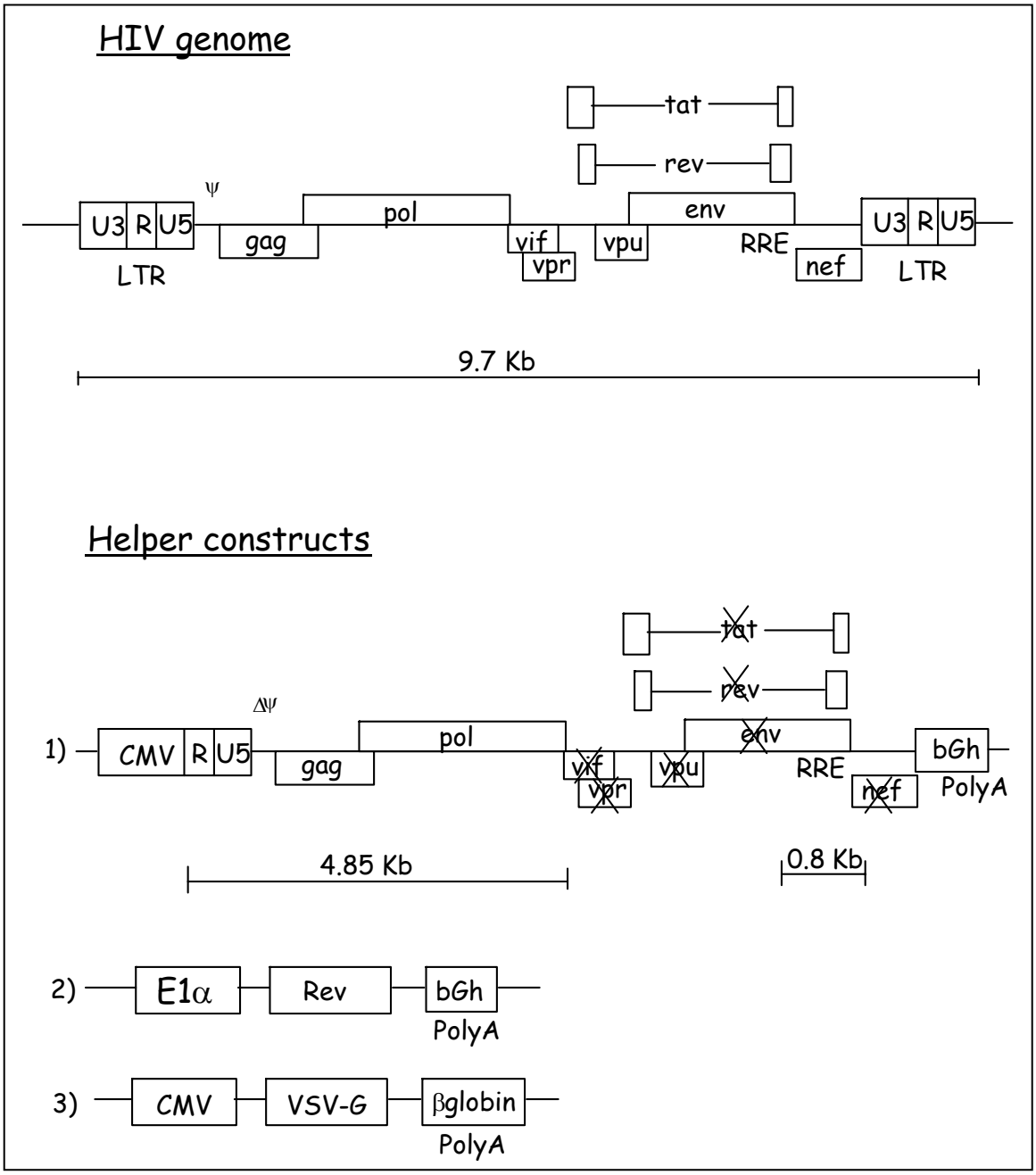
Lentiviral vectors and containment issues.

Nikunj Somia, University of Minnesota.

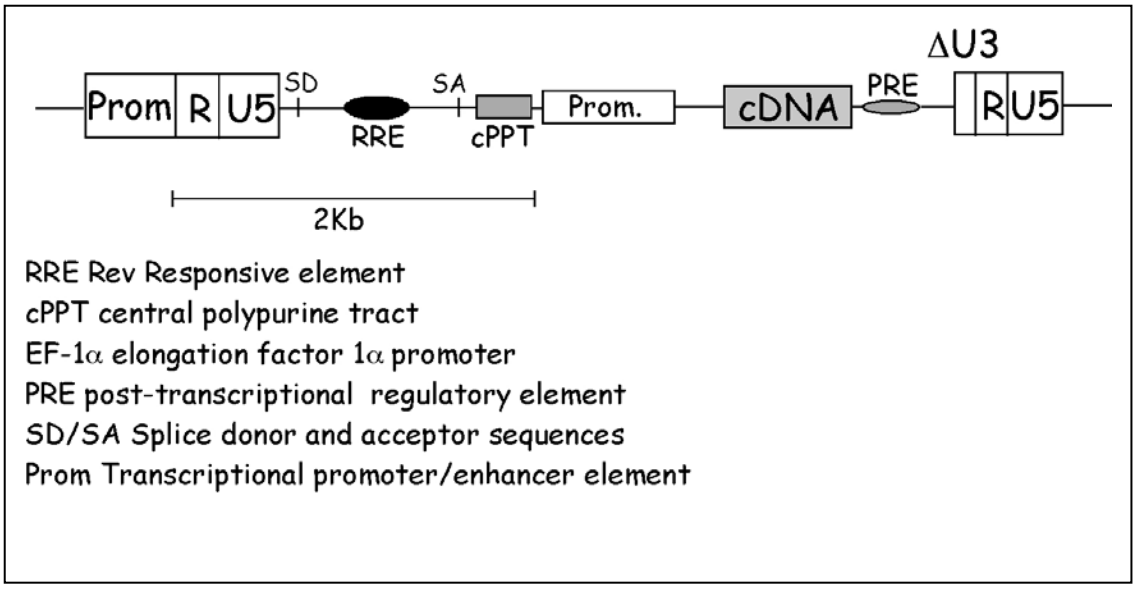
The technology for the production of lentiviral vectors has disseminated widely (with commercial enterprises selling "kit" forms to produce vectors i.e. HIV-1 based vectors from Invitrogen, FIV and HIV vectors from System Biosciences) and the general availability of packaging systems from academic laboratories. This poses a problem for IBC's around the country as to the level containment to impose on investigators that wish to use this technology both cell culture and in animal models of disease.

A. Overview of the System.



The full length HIV-1 genome is illustrated at the top of the figure. Below that are illustrated the current "third generation" helper plasmids used for viral vector production. The first plasmid (1) codes for the *gag* and *pol* genes of HIV. This plasmid is multiply deleted, to reduce the risk of generating wild-type HIV-1 by recombination. The deletions have resulted in only 5.6 Kb of the full length 9.7 Kb remaining in the helper plasmid (58%). Notably this plasmid is also deleted for all the accessory genes of HIV-1, which have been documented to be necessary for the pathology caused by the wild type virus. The only accessory protein needed (for transport of full length transcripts out of the nucleus) is Rev. This is provided by the second plasmid (2). Finally the third plasmid (3) codes for the G protein of Vesicular Stomatitis Virus, and serves as an envelope for the viral vector, which is a commonly used envelope for retroviral vectors.

Vectors:



The figure illustrates a typical generic vector based in the third generation HIV-1 vector system. Of note is the deletion of the U3 in the 3' LTR. This modification leads to no functional promoters for HIV-1 in target cells.

A. Safety considerations:

1) Potential of replication competent lentivirus (RCL) being generated during production of the vector

2) Potential of productive infection of animals injected with a RCL in a vector preparation

3) Potential of viral vector mobilization

4) Vector / Virus shedding for animals

5) Accidental injection of vector preparation into personnel

6) The importance of the gene in the vector.

B. Experience to date.

1) The experience with MLV based retrovirus vectors is informative and useful for considering the generation of replication competent retrovirus (RCR) during production of retroviral vectors (this was covered in depth at the September 19th 2002 RAC meeting - retroviral packaging cell lines). In summary, although RCR has been observed from MLV vectors, judicious choice of the helper plasmid and vector – with a minimum sequence overlap between the plasmids can control the problem. The optimization of helper and vector sequences for MLV based vectors is facilitated by assays for RCR and wild-type HIV. These assays can be conducted in a BL2 tissue culture facility and replication competent MLV can be used as a positive control. For HIV-1 based vectors a number of assay have been adapted to study the generation of RCL. The most widely used include i) monitoring for the spread of virus in target cell population by assaying for the presence of capsid protein (p24) in the cell culture. The rationale is that if RCL is present p24 levels will increase over time ii) vector mobilization assays. A cell line with an HIV-1 vector carrying a reporter gene (tester cell) is infected with the test supernatant, and after some time in culture the supernatant from the tester cells is used to infect naïve cells. These cells are assayed for transfer of the vector reporter. The rationale is that if RCL was present it will mobilize the vector. iii) PCR based approaches to assay for the transfer of *gag* and *pol* sequences to target cells. The rationale is that any RCL must have *gag* and *pol* genes in order to propagate and hence these should b serially transferred to subsequent naïve cultures. To date Dr. Inder Verma's laboratory (with 9-10 years experience with these vector systems) has never detected an RCL event (personal communication) using p24 and vector mobilization assays. Indeed the National Gene Vector Laboratories (NGVL) directed by Dr. Kenneth Cornetta has never observed a RCL event (personal communication). The NGVL has tested vectors and helper plasmids from various sources. The laboratory has produced over 60 liters of HIV-1 vector and not detected RCL. Using the *gag* and *pol* PCR test the NGVL has on rare occasions observed transfer of sequence but this was not propagated on subsequent cultures and therefore cannot be classified as an RCL event. The NGVL has never observed the transfer of envelope (VSVG) sequences to target cells.

2) Potential of productive infection of animals injected with a RCL in a vector preparation. What are the implications of infection of animal models with a RCL in the rare (and so far undetected) event of RCL being generated? This consideration should be informed by the lack of productive HIV-1 replication in small animal models. Hence no mouse or rat models are available to study HIV-1 replication. Indeed HIV-1 replication is also inhibited in monkeys. Infection of these cells is possible only with high titers of viral vectors. The conclusion from these observations is that experiments using small animal models with HIV vectors have an intrinsic safety component in species restrictions to productive HIV-1 infection. An exception to this is experiments where human cells

infected with HIV-1 (and vectors) are injected into immunodeficient mice. These do have the potential to amplify any rare RCL event.

3) Potential of viral vector mobilization. This would require that small animal models or cell cultures are also infected with a virus that can mobilize the vector. Due to restrictions to HIV-1 replication in small animals (mice) the virus would have to be a retrovirus that can replicate in mice and be able to cross package the HIV-1 vector genome. Although this may happen (for example MLV cross packaging HIV-1 genomes) this is very inefficient, not readily detected in culture systems, and unlikely to generate a systemic infection the animals.

4) Vector / Virus shedding for animals. Vector shedding from animals is more a concern than virus shedding (see above) unless the vector contains an insert of concern (i.e. oncogenic). The danger would be through direct contact with skin / mucous membranes etc. which is also true for transmission of other microbes from animals to the investigator.

5) Accidental injection of vector preparation into personnel. Most vectors that are utilized, reporters or disease correcting genes would not pose a threat, and the result would presumably be local inflammation at the site of injection with some dissemination of the vector. The total number of particles injected will be a factor in the immune reaction observed in the patient to the vector particles. Presumably this will be limited by the amount of virus that is being administered to a small animal model and these have not demonstrated toxicity in mice. Pre-clinical toxicity studies for HIV-1 vectors will inform us as to what is an upper dose of an accidental injection that poses a serious concern.

6) A special case can be argued for vectors that have an oncogenic gene in the vector. This may constitute the “first hit” in an oncogenic progression for an investigator accidentally infected with a viral vector.

Summary and recommendations.

Given the lack of any RCL detection using the third generation HIV-1 vectors I would recommend that vector production be allowed under BL2 tissue culture practices. The total lack of productive HIV-1 infection in small animals already affords a level of protection and I would recommend that animals injected with vectors (after suitable precautions have been taken to counter vector shedding during injections etc) can be housed in normal ABL1 facilities. The exception being mice that have human cells injected that may allow replication of HIV-1. In this case, the institutional IBC should access if the cells can support productive HIV-1 (or RCL) spread. I recommend similar containment practices for other lentiviral vectors (FIV, SIV, EIAV etc) provided that the design is analogous to the third generation HIV-1 vectors.