## Centre for AIDS Reagents.

## Data Sheet

**NAME:** pSHIV-89.6 3’

**REPOSITORY REFERENCE :** ARP2123

**PROVIDED:** 5.78 µg plasmid DNA. Propagate in *E. coli* XL1-Blue grown at 30°C.

**DESCRIPTION:** SHIVs are chimeric simian/human immunodeficiency viruses composed of SIVmac239 modified to include HIV-1 env and the associated auxiliary HIV-1 genes tat, vpu, and rev. The SHIV-89.6 3' construct encodes the HIV-1 portions of the chimeric virus, cloned into the pBluescript II KS(+) vector (Stratagene). The insert size is 5.9 kb, and the plasmid size is 8.9 kb.

**CHARACTERISTICS:** The HIV-1 env sequences were derived from the macrophage-tropic isolate HIV-1 89.6. Infectious virus can be generated by ligating pSHIV- 89.6 5' and pSHIV-89.6 3', and transfecting CEMx174 cells with the resulting construct.

**STORAGE:** -70°C

**SOURCE:** Dr. Joseph Sodroski (Courtesy of the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.)



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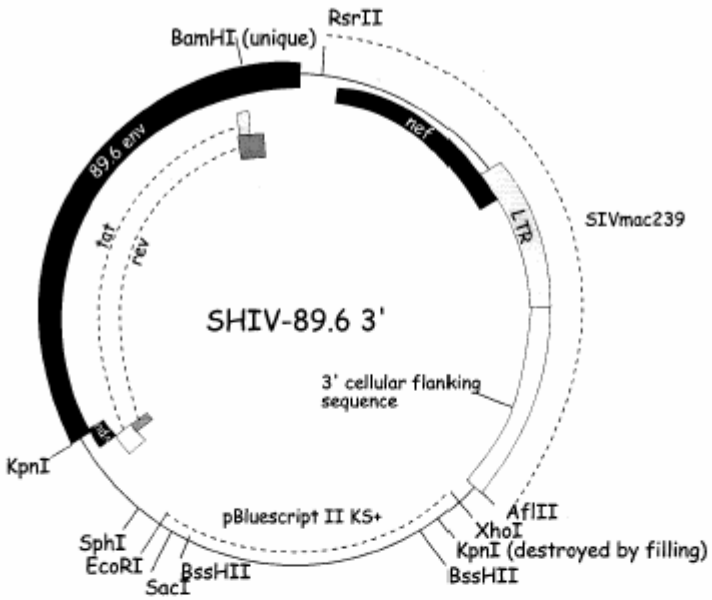
**REFERENCE:**

Karlsson GB, Halloran M, Li J, Park IW, Gomila R, Reimann KA, Axthelm MK, Iliff SA, Letvin NL, Sodroski J. Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4+ lymphocyte depletion in rhesus monkeys. *J Virol* **71**:4218-4225, 1997.

**ACKNOWLEDGEMENTS:**

Publications should acknowledge the donor of the reagent and the Programme EVA Centre for AIDS Reagents. Suggested wording can be found on our website at http://www.nibsc.ac.uk/spotlight/aidsreagent/index.html in the "Acknowledgements" section.
Please also ensure that you send us a copy of any papers resulting from work using reagents acquired through CFAR (this can be electronically or as a paper copy)




1. EcoRI-XhoI fragment of HXBc2 (5743-8897) cloned into pBluescript II KS+

2. RsrII and AflII sites introduced into HXBc2:

```
8796                          8819
   GATGGGTGGCAAGTGGTCAAAAAG
     C  ACC            TT  G
     RsrII             AflII
```

3. RsrII site introduced into SIVmac239 nef-open:

```
9313          9327
   TGAACTGACCTACCT
        G     G
        RsrII
```

4. RsrII-AflII fragment of SIVmac239 nef open inserted into the modified HXBc2 subclone.

5. SphI site and last two codons of SIV vpr were introduced into HXBc2 sequence:

```
5808                5822
   TACTCGACAGAGGAG
      GC TGCT T
```

# TRANSFECTION OF CEMX174 CELLS FOR SIV OR SHIV PRODUCTION

## DIGESTION

1. Digest 5 µg each proviral half with the appropriate restriction enzymes in a total volume of 80 µl. Remove a 5 µl aliquot and run a gel to make sure digestion has gone to completion.

| | |
|---|---|
| SHIV-KB9 | Cut the 5' half clone with SphI + XhoI |
| Digest: | Cut the 3' half clone with SphI + NotI |
| | |
| SHIV-89.6 | |
| Digest: | Cut the 5' half clone with SphI + ClaI |
| | Cut the 3' half clone with SphI + AflII |

Phenol/chloroform extract the digested DNA once. Precipitate with ethanol using standard procedures.

Resuspend pellets in 20 µl dH2O and set up ligations in a final volume of 50 µl, using the total 20 µl volume of each half. Ligate for at least 3 hours at 17°C.

## TRANSFECTION

1. Prepare 2M Tris buffer, pH 7.3, and 50 mM Tris buffer, pH 7.3. Filter sterilize.

2. Prepare DEAE-dextran at 25 mg/ml in the 50 mM Tris buffer, pH 7.3 (0.25 g DEAE-dextran in 10 ml). Filter sterilize.

3. Prepare DME/DEAE by adding 1.25 ml of the 2M Tris buffer, pH 7.3, and 0.25 ml of the 25 mg/ml DEAE-dextran solution into 48.5 ml of serum-free DMEM.

4. Wash CEMx174 cells (use 5 x 106 cells for each transfection) twice in serum-free DMEM.

5. Add 1.4 ml of the DME/DEAE mix to each 50 µl ligation mix. Vortex gently to mix well.

6. Resuspend the cell pellet in the 1.4 ml DNA/DEAE/DMEM mix.

7. Incubate for 1 hour at 37°C.

8. Centrifuge the cells. Wash once in serum-free DMEM, and once in serum-free RPMI 1640.

9. Resuspend the cells in 8-10 ml RPMI 1640 containing 10% fetal bovine serum and pen-strep. Transport the cells to a containment suite, if the procedure was not already performed there.

10. Monitor virus growth in the culture every two days (split the cells as needed at the same time). For SHIVs, virus is usually detected after 4-5 days, and will peak in the culture about 7-10 days. SIV is usually a little quicker.

## PLASMID DNA

DNA from the plasmids containing the proviral halves can be grown in XL1-Blue bacteria. The bacteria should be grown at 30°C for better yield in DNA preparation.