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DATA SHEET

Reagent:	SHIV 89.6P 5' Partial Molecular Clone (pSHIV-89.6 5')
Catalog Number:	4130
Lot Number:	041243
Release Category:	E
Provided:	5 µg of dried purified DNA stabilized in DNAstable *PLUS*
Cloning Vector:	pBluescript II KS(+) Ampicillin resistant
Cloning Site:	HindIII/SphI cloning site The size of the insert is 6491 bp.
GenBank:	U89134
Host Strain:	Plasmids can be propagated in STBL2 cells and grown at 37°C. Larger plasmids may benefit from growth at 30°C. This construct may also be grown in other competent cells.
Description:	A partial SHIV 89.6 5' chimeric molecular clone.
Special Characteristics:	This construct is 9671 bp including the insert. The source of this chimeric molecular clone is derived from the SHIV-89.6 virus. The SHIV-89.6 virus expresses the gag, pol, vif, vpr, vpx, and nef proteins of SIVmac239 (*nef* open) and the tat, rev, vpu, and env proteins of HIV-1. The *env* gene is derived from a cytopathic primary HIV-1 isolate, 89.6, which uses both CCR5 and CXCR4 as co-receptors. The *tat*, *rev*, and *vpu* genes are derived from the HIV-1 HXBc2 isolate. The 5' clone consists of the sequences from the SIVmac239 clone and contains the *gag*, *pol*, *vif*, *vpx*, *vpr*, and *tat* genes as well as the 5' LTR.

Infectious virus can be generated by ligating the SphI-digested proviral halves, pSHIV-89.6 5' (Cat# 4130) and pSHIV-89.6 3' (Cat #4131), and transfecting CEMx174 cells with the resulting construct.

Contributor provided plasmid map

Sequence file lot 041243

This reagent is currently being provided as dried purified DNA stabilized in DNAstable *PLUS*. Please see the notice for additional information and the protocol for reconstitution of dried DNA reagents. Dried DNA Notice

Recommended Storage:

Keep the reagent at room temperature in a dry storage cabinet or in a moisture barrier bag.

Contributor:

Dr. Joseph Sodroski

References:

G. B. Karlsson, M. Halloran, J. Li, I. W. Park, R. Gomila, K. A. Reimann, M. K. Axthelm, S. A. Iliff, N. L. Letvin and J. Sodroski. (1997). Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4+ lymphocyte depletion in rhesus monkeys. J Virol, 71(6), 4218-25. PUBMED

J. Li, C. I. Lord, W. Haseltine, N. L. Letvin and J. Sodroski. (1992). Infection of cynomolgus monkeys with a chimeric HIV-1/SIVmac virus that expresses the HIV-1 envelope glycoproteins. J Acquir Immune Defic Syndr, 5(7), 639-46. PUBMED

NOTE:

Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: SHIV 89.6P 5' Partial Molecular Clone (pSHIV-89.6 5') from Dr. Joseph Sodroski (cat# 4130)." Also include the reference cited above in any publications.

Recipient must not use or incorporate the reagent for commercial purposes.

Research Chart:

Transfection of CEMx174 Cells for SIV or SHIV Production

Digestion

1. Digest 5 ìg 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 Digest:

   Cut the 5' half clone with SphI + XhoI
   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

2. Phenol/chloroform extract the digested DNA once. Precipitate with ethanol using standard procedures.
3. Resuspend pellets in 20 µl dH2O and set up ligations in a final volume of 50 µl, using the total 20 ìl volume of each half. Ligate for at least 3 hours at 17ºC.

Transfection

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

- 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.
- Wash CEMx174 cells (use 5 x 106 cells for each transfection) twice in serum-free DMEM.
- Add 1.4 ml of the DME/DEAE mix to each 50 µl ligation mix. Vortex gently to mix well.
- Resuspend the cell pellet in the 1.4 ml DNA/DEAE/DMEM mix.
- Incubate for 1 hour at 37ºC.
- Centrifuge the cells. Wash once in serum-free DMEM, and once in serum-free RPMI 1640.
- 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.
- 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.

Last Updated: September 19, 2018