Supporting Information

Borenstein and Frenkel 10.1073/pnas.0908504106

SI Text

Transfection of SupT1 Cells with HHV-6A-BAC. One day prior transfection, the growth medium was replaced by fresh medium and cells were kept at low density to avoid stress. The transfection was by applying 6 μg HHV-6A-BAC DNA to 0.5 $\times$ 10^6 cells, using the MP-100 microporator device, in a 100-μL gold-tip, as recommended by the manufacturer. The electroporation parameters were two pulses of 1,100 V with pulse width of 30 ms.

Transfection of 293T Cells with HHV-6A-BAC. The 293T cells were propagated in DMEM, 10% (FCS), 2 mM L-glutamine, and pen-strep-nistatin. The transfection was performed employing Lipofectamine (Invitrogen) by the manufacturer's instructions. For stable transfection, the cells were maintained in the medium above plus 1 μg puromycin/mL (Sigma Chemical).

Pulse-Field Gel Electrophoresis. DNA samples were loaded into gel wells along with DNA size ladder: 15–300-kb DNA ladder (Midrange I, New England BioLabs) and 0.25–10-kb ladder (Perfect Plus, 1 kb EURx). Pulse-field gel electrophoresis was conducted using the Counterclamped Homogeneous Electric Field (CHEF) apparatus (Bio-Rad), in 14 °C. Running parameters were 18 h, 6 V/cm, 120°. Switch times were 1–25 s. The gel was stained with 1 μg/mL EtBr, visualized with UV light, and photographed.

Southern Blot Analyses of HHV-6A-BAC with the pac-2-pac-1 Junction Probe. The HHV-6A-BAC DNA was analyzed by blot hybridizations using a 130-bp probe with sequences of the pac-2 and pac-1 signals. This probe detects sequences from the termini as well as DRR-DRL junctions. The pac-2-pac-1 probe was generated by PCR of total HHV-6A (U1102) DNA as the template with the primers: pac-1 starting at 52 bp anti-sense: 5′ccccccctttttttagccccccggg′3 and pac-2 starting at 159,244 bp sense: 5′cccccaacgcgcgcgcgcac′3.

Direct Cloning of the Intact HHV-6A DNA into BAC. The pIndigoBAC-5 was modified to abolish one *Not*I site and the *Sfi*I site (Fig. S1*B*). The mutation was introduced by PCR using the primer pairs 5′gaacccctgcgAccgcccAggccgtc′3 and 5′gacggccTgggcggTcgcaaggggttc′3 (capital letters denote the mutations). The resultant pNF1254 clone contained a single *Not*I site at position 2 of the original BAC-5 vector and no *Sfi*I sites. An ampicillin/puromycin (amp/puro) cassette was prepared by PCR, employing the pMSCV plasmid (Clontech). PCR of the puro segment was done (Fig. 1*C*) employing sense and anti-sense primer pairs, with a *Not*I site at the 5′ terminus and a *Mlu*I site at the 3′ terminus 5′ATAAGAATgcggccgcCGAATTCTACCGGGTAGGG′3 and 5′ACGacgcgtTGCCAAACCTACAGGTGG′3 (in this and other sequences below lowercase letters correspond to the restriction enzyme sites). PCR of the amp segment was done employing a primer pair containing a *Not*I site at the 5′ terminus and *Mlu*I site at the 3′ terminus, corresponding to 5′ATAAGAATgcggccgcCTTAGACGTCAGGTGGCAC′3 and 5′ACGacgcgtGGTCTGACAGTTACCAATGC ′3. The puro and the amp PCR products were digested with *Mlu*I and then ligated to form the amp/puro cassette, flanked by the *Not*I sites (Fig. S1*C*). The cassette digested with *Not*I was cloned into the *Not*I of pNF1254, yielding the pNF1255 plasmid (Fig. S1*D*). The EGFP gene was made by PCR from pEGFP-C3 (Clontech) employing primer pairs with a *Bst*EII site at the 5′ terminus: TTGggttaccCCTGCAGCCCTAATAGTAATCA and 5′TTGggttaccCGCTTACAATTTACGCGTTAAG′3. The PCR fragment was blunted by the Klenow reaction and ligated into the *Hpa*I site of pNF1255, generating pNF1266 (Fig. S1*E*). Finally, a DNA fragment with *Bam*HI-*Sfi*I-LoxP and *Hin*dIII sites was produced by annealing the oligonucleotides: 5′gatcCTTCTTggccacctcggccTTGCTTGGCGCGCCGTataacttcgtatagcatacattatacgaagttatTT′3 and 5′AGCTAAataacttcgtataatgtatgctatacgaagttatACGGCGCGCCAAGCAAggccgaggtggccAAGAAG′3 (the lowercase letters denote restriction enzyme sites and LoxP sites). The oligomer was ligated with the pNF1266 plasmid, cleaved with *Bam*HI and *Hin*dIII. This generated the BAC-amp-puro-GFP (pNF1267) vector (Fig. S1), containing the *Sfi*I site matching the *Sfi*I site of HHV-6A DNA and LoxP sequence. The pNF1267 vector was digested with *Sfi*I, dephosphorylated, and ligated with the *Sfi*I-cleaved HHV-6A DNA prepared by the Hirt procedure. The ligation produced several colonies tested by PCR.

Screening of the HHV-6A-BAC Clones. The BAC clones were transferred into 96-well plates containing 200 μL LB, supplemented with 7% glycerol. After 2 h incubation, they were screened by PCR, employing six pairs of test primers: (*i*) primers for BAC (B) positions 2,189–2,573, expected to yield a 384-bp PCR product. The BAC primers corresponded to sense: 5′TGCCTGCAAGCGGTAACGA′3 and anti-sense 5′ACACCTTCTCTAGAACCAGC′3. (*ii*) A set of primers from the left part of the genome, positions 15,000–15,602, yielding a 602-bp PCR product. The primer sequences were 5′CCCCATTCAACATCCTATTGA′3 and 5′CCGGAAAGACCATTGCCG′3. (*iii*) A set of primers corresponding to the middle part of the genome, positions 75,208–75,536, yielding a 328-bp PCR product. The sequences of the primers were 5′GCGCTGTACATTTTTCCAG′3 and 5′CTTCAC CGTCTCTGAAGCA′3. (*iv*) A set of primers corresponding to the right part of the genome, positions 134,263–134,591 and yielding a 328-bp PCR product. The sequences of the primers were 5′TTCTCCAGATGTGCCAGGGAAATCC′3 and 5′CATCATTGTTATCGCTTTCACTCTC′3. (*v*) Primers of the amp/puro cassette, yielding a 2,268-bp PCR product. The primers corresponded to 5′ATAAGAATgcggccgc CTTAGACGTCAGGTGGCAC′3 and 5′ATAAGAATTTCTACCGGGTAGGG′3. (*vi*) Primers for the detection of the EGFP cassette, yielding a 1,425-bp PCR product. The primer sequences were 5′ttgggttaccCCTGCAGCCCTAATAGTAATCA′3 and 5′ttgggttaccCGCTTACAATTTACGCGTTAAG′3. All PCR tests gave the expected sizes and the clones were designated HHV-6A-BAC (pNF1268), carrying the intact viral genome.

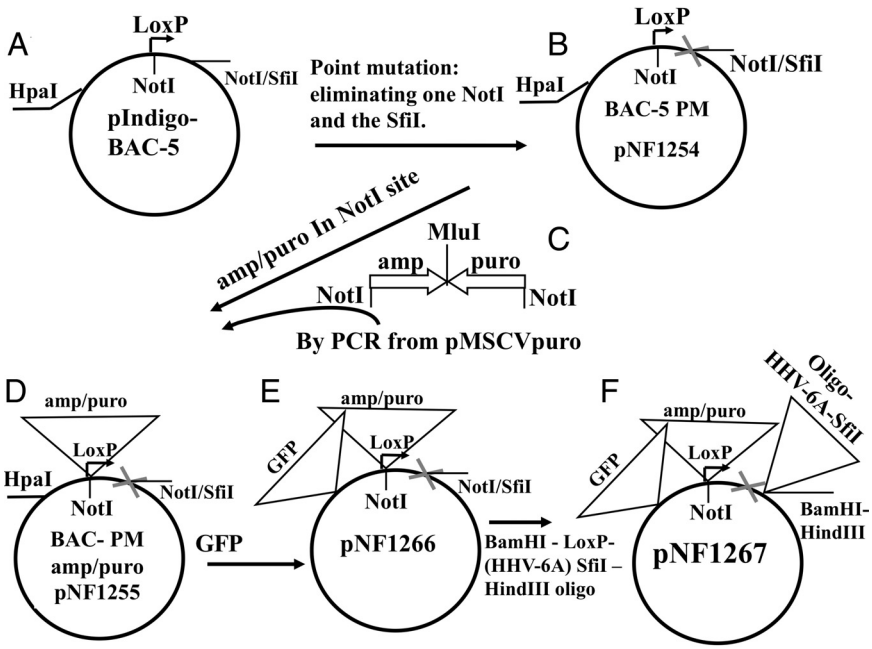

Fig. S1. Derivation of the BAC clone pNF1267. The pIndigoBAC-5 was modified as follows: (*A*) Sites in the original pIndigoBAC-5, purchased from EPICENTRE Technologies. (*B*) One of the two *Not*I sites and the *Sfi*I site were eliminated by point mutations. (*C*) The amp/puro cassette was synthesized from pMSCV-puro by PCR of the amp and puro genes with primers containing the *Not*I and *Mlu*I sites. The amp and puro genes were then linked at the *Mlu*I site, producing a cassette flanked by *Not*I sites. (*D*) The amp/puro cassette was inserted into the remaining single *Not*I site. (*E*) The GFP gene driven by the CMV promoter was introduced into the *Hpa*I site of pNF1255, yielding pNF1266. (*F*) An oligonucleotide containing the LoxP sequence, *Sfi*I site matching the HHV-6A *Sfi*I site, and *Bam*HI and *Hin*dIII complementing sequences was ligated into the *Bam*HI-*Hin*dIII sites of the PNF1266. This produced the BAC-amp-puro-GFP, pNF1267 vector with the desired sites.