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Summary

Improvements in methodologies to recapitulate and study particular biological functions of the papillomavirus life cycle have led to great advances in our knowledge of these viruses. Described in this chapter are techniques that allow low-copy and high-copy replication of full-length human papillomavirus (HPV) genomes, as well as assembly of virus-like particles, in *Saccharomyces cerevisiae* (yeast). This system has several distinct advantages that make it an attractive complement to the well-established raft-culturing system. First, yeast are inexpensive, rapid, and simple to culture in the lab. Second, they provide an ever-widening array of genetic tools to analyze HPV functions —most recently notable, the yeast ORF-deletion library. Third, yeast provide a potentially high-efficiency means to produce large quantities of infectious virus in a short time frame. Fourth, assembly of HPV virus in yeast allows encapsidation of mutant genomes, since previous studies have shown that no viral ORF is required for replication of full-length HPV in yeast.

1. Introduction

The advance represented by keratinocyte raft culture was a breakthrough that greatly improved our ability to study the molecular details of the human papillomavirus (HPV) life cycle. This technology has permitted analysis of replication of wild-type or mutant genomes within the appropriate biological context of a differentiating epithelium ([1,2] and *see* Chapters 12, 13, and 14). In further studies, Michelle Ozbun's lab has succeeded in utilizing raft culturing as a means to produce significant quantities of infectious virus (3). However, impediments still remain in understanding certain aspects of the viral life cycle. In particular, the precise *cis* and *trans* packaging requirements of HPVs, the details of viral assembly, virion attachment, uncoating, and egress are less well characterized in HPVs than in other viral systems. We also lack efficient technologies for encapsidating mutant genomes for use in infection studies, something that can be achieved with a number of other viral systems. Previous studies have established that HPVs can replicate in yeast (4-6). Further developed methodologies are described here, which allow not only replication, but also the amplification and encapsidation of target HPV genomes in yeast. Previous work has established that L1-L2 pseudoviruses can readily be formed in yeast and that these are capable of encapsidating partial HPV genome constructs (7). These particles have been shown to be capable of transduction of epithelial cells. The methods described here allow packaging of actively replicating, full-length HPV genomes, and the resultant pseudovirus particles are of authentic morphology.

2. Materials

2.1. DNA Cloning

1. Competent DH5α *Escherichia coli* (Invitrogen).

[14]The efficiency of recovery of packaged genomes is approx 3×10^7 DNase-resistant DNA-containing units (DCUs) isolated from approx 2×10^8 cells in a 25-mL culture (from yeast containing 50 copies per cell of the HPV genome). This results in approx 1 DCU per seven yeast cells.

2. Plasmids containing full-length HPV (described in **refs.** 1 and 4).
3. Plasmids containing a yeast selectable marker and promoter, such as *Ura3*, *Trp1*, *Leu2*, or *His3* (American Type Culture Collection [ATCC]).
4. Primers to amplify the yeast selectable markers and promoter and viral open reading frames (ORFs).
5. Taq polymerase and T4 DNA ligase for DNA manipulations (Promega).
6. Calf-intestine alkaline phosphatase (CIAP; Promega).
7. Restriction enzymes (New England Biolabs).
8. Luria-Bertani (LB) broth (Becton, Dickinson Co.).
9. Ampicillin.
10. Qiaquick kit (Qiagen).
11. Bacterial plasmid miniprep kit (Qiagen).
12. Agarose.
13. Plasmids for selective expression of viral ORFs, i.e., containing a galactose-inducible promoter (BD Biosciences or Invitrogen).
14. YPH500 or any yeast strain with suitable selectable markers (ATCC).

2.2. Transformation of Plasmids into Yeast

1. Yeast selective and nonselective media (Becton, Dickinson Co.).
2. Frozen-EZ transformation kit (Zymo Research, Orange, CA).

2.3. DNA Isolation From Yeast

1. Yeast DNA isolation buffer: 10 m*M* Tris-HCl (pH 8.0), 100 m*M* NaCl, 2% Triton X-100, and 1% sodium dodecyl sulfate (SDS).
2. Acid-washed glass beads (400 nm; Sigma).
3. Yeast miniprep kit (Zymo Research, Orange, CA).
4. Speed-vac (Savant).

2.4. DNA Replication Assays of Plasmids Recovered From Yeast

1. *Dpn*I (New England Biolabs).
2. Southern blot reagents (*see* Note 1).
3. Rediprime DNA labeling kit (Amersham).
4. Phosphorimager (Molecular Dynamics).

2.5. RNA Isolation From Yeast

1. DNase (RNase free; Promega).

[1]In general, the techniques for Southern analysis of yeast DNA are the same as with any other source of DNA. One exception is that DNA preparations from yeast often have a pink, insoluble pigment that can affect gel loading and the electrophoretic migration of DNAs. This material reduces the clarity of the resulting Southern blots. A simple solution to this problem is to microfuge the DNA preparations at 14,000*g* for 5 min, recover the clarified supernatants, and use these to load onto a gel.

2. Buffer A: 50 m*M* sodium acetate (pH5.2), 10 m*M* EDTA, 1% SDS.
3. Northern blot reagents.
4. RNA markers, 0.28-6.58 kb (Promega).

2.6. HPV Pseudovirion Isolation From Yeast

1. Virion isolation solution 1: 1 *M* NaCl, 50 m*M* $NaPO_3$ (pH 8.0).
2. Virion isolation solution 2: 50 m*M* NaCl, 50 m*M* $NaPO_3$ (pH 7.4) (*see* Note 2).
3. Phenylmethylsulphonyl fluoride (PMSF) or protease inhibitor cocktail (Sigma)

2.7. Analysis of Encapsidation by DNase Treatment

1. DNase I (Promega).
2. DNase buffer: 100 m*M* NaCl, 10 m*M* $MgCl_2$, 10 m*M* Tris-HCl (pH 7.9).
3. DNase stop buffer: 100 m*M* NaCl, 100 m*M* EDTA, 1% SDS, 20 m*M* Tris-HCl (pH 7.9).

3. Methods

The methods described here outline (1) the construction of HPV genome vectors that are competent for replication in yeast, and vectors for expression of HPV ORFs, (2) analysis of episomal replication and trans-acting effects of E2 in yeast, and (3) assembly and isolation of HPV pseudoviruses from yeast.

3.1. HPV-Genome Constructs

The construction of HPV genome vectors for yeast is outlined below. The general approach is to insert a yeast nutritional marker, such as *Ura3*, *Trp1*, *Leu2*, or *His3*, into a location within the genome sequence, such that functions to be studied are not affected. In the example described here, *Ura3* gene was placed between the L1 ORF and the LCR of HPV 16 or HPV 31 to create pPA103 and pPA106, respectively (Fig. 1A).

3.1.1. Cloning Methods—Standard cloning methods are used to subclone a yeast nutritional marker into the HPV genome. They are described in outline here.

1. Locate a suitable cloning site within the HPV genome (*see* Note 3).
2. Design primers to amplify the chosen nutritional marker along with its constitutive promoter. Engineer appropriate restriction sites into the primers for the purpose of cloning the marker into the chosen restriction site in the HPV genome.
3. Polymerase chain reaction (PCR)-amplify the marker gene, ethanol-precipitate the DNA, and digest both the PCR product and the HPV DNA with the chosen restriction enzyme.
4. Phosphatase the vector DNA using CIAP for 30 min at 37°C in the provided buffer.
5. Gel-purify both DNAs using the Qiaquick kit.

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[2]After isolation of pseudovirions, they can be analyzed immediately by Southern or Western blots or stored at 4°C. If the pseudovirions are to be stored, it is a good practice to include 1 m*M* PMSF or a protease-inhibitor cocktail to protect the pseudovirions from degradation.
[3]An important consideration when designing the HPV/yeast vector is where to clone the nutritional marker. This depends upon the type of studies to be done. For the examples given in this chapter (pPA103 and pPA106; Fig. 1), cloning sites were chosen that did not disrupt any viral ORF, thus allowing the potential for appropriate gene expression to occur. Removal of the vector sequence prior to transformation into yeast leaves a minimum of foreign sequence (the marker) and allows the replication-competent genomes to be of packageable size.

6. Ligate DNAs in a molar ratio of 3:1, insert to vector for 2 h at room temperature, and transform into *E. coli* DH5α.
7. Screen colonies for clones that contain inserts by digestion of miniprep DNAs with the chosen restriction enzyme.

3.2. HPV-ORF Expression Vectors

The pPD2-16E2 vector, diagramed in Fig. 1B, achieves E2 expression via a phosphate-dependent promoter (pho5). The pho5 promoter yields a low to moderate level of E2 expression, which is the desired level, since over-expression of E2 is known to lead to G2/M arrest in yeast (*Schizosaccharomyces pombe*) (8). The pPD2-16E2 plasmid also contains a yeast 2-μ origin, the *Leu2* biosynthetic gene, a *coli* 1 origin, and an ampicillin marker. A complete description of the construction of this plasmid is given in (9). For expression of HPV-16 L1 and L2, a bidirectional galactose-inducible promoter was used; the vector is referred to here as pL1L2 (Fig. 1B). The L1 ORF was generated by PCR amplification from pEF399, which contains HPV 16 (W12E) (10). The L2 ORF was amplified from a codon-optimized version created by Leder et al. (11).

3.3. Yeast Strains

A haploid yeast strain such as YPH500 (12) (*MAT-α Ura3-52 lys2-801 ade2-101 trp1-63 his3-200 leu2-1*) can be used. We have witnessed identical results of HPV replication with different yeast strains, such as HF7 (*MAT-a, Ura3-52, his3-200, lys2-801, ade2-101, trp1-901, leu2-3, 112, gal4-542, gal80-538, LYS2::GAL-HIS3, URA3::(GAL4 17-mers)3-CYC1-lacZ; Clontech).* Therefore, the choice of yeast strain is limited only by the availability of nutritional markers (*see* Note 4).

3.4. Transformation of Plasmids into Yeast

Approximately 200 ng of plasmid DNA is used to transform YPH500 yeast. The method of greatest simplicity utilizes the Frozen-EZ transformation kit, and instructions are provided with the kit (*see* Note 5). Yeast transformations are plated on selective media and analyzed for colony formation after 3 d. An example of transformation results is shown in Fig. 2A. Note that colonies are formed only when the HPV-16 genome is present, and that *Ura3* (puc18 Ura) itself is insufficient to allow colony formation. In most cases, sequential transformation is used to create yeast strains containing multiple plasmids (*see* **Notes** [4], [6]).

3.5. DNA Isolation From Yeast

1. Inoculate yeast harboring HPV plasmids from an individual colony into a 5-mL liquid culture of nutritionally selective medium and grow at 30°C with vigorous shaking.
2. Use the entire 5-mL culture to inoculate a 25-mL culture and grow to yield an OD_{600} over 1.0. Typically, 2-5 × 10^8 cells are harvested per 25-mL culture.

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[4]It is also possible to mate yeast containing different plasmids, and this is often a very convenient and efficient method to create the desired strain. For this purpose, one needs only to choose a strain of the opposite mating type but genotypically identical in terms of the auxotrophic mutations. For example, YPH499 is *Mat-a* and YPH500 is *Mat-*α (12), but they are otherwise genotypically identical, and are therefore good candidates for mating (*see* **Subheading 3.3.** for genotype information). In the lab, we have found that diploid strains containing the multiple HPV plasmids described here appear to have even better stability characteristics than haploid strains. Details regarding standard yeast mating techniques can be found in the *Current Protocols in Molecular Biology* manual (14).

[5]An alternate technique for transformation of plasmids into yeast is by means of the standard LiAc and PEG 8000 method, as described by Schiestl et al. (17). An important tip here is to use yeast that are actively dividing in mid-log phase for optimal transformation efficiency.

[6]Previous results in our lab have demonstrated that HPV genomes replicate very stably in yeast (4), even in the presence of additional plasmids, such as those containing E2 (pPD2 16E2) and L1 and L2 ORFs (pL1L2). However, it is still advisable to create glycerol stocks of each yeast strain and begin each experiment with fresh, actively growing yeast directly from these glycerol stocks. This minimizes the chances of unwanted recombination, mutations, or changes in plasmid copy number from occurring.

3. Pellet the cells by centrifugation in a table-top centrifuge at 2000*g* and resuspend them in 600 μL of yeast DNA lysis buffer.
4. Add 300 μL of 400-nm acid-washed glass beads. Add 600 μL of phenol and vortex the mixture for 1-2 min.
5. Recover the supernatant by brief centrifugation in a microfuge and transfer the supernatant to a new tube. Precipitate the DNA by addition of 2.5 vol of 100% ethanol and incubating at -20°C for at least 10 min.
6. Recover the DNA by centrifugation at 14,000*g* for 10 min at 4°C in a microfuge.
7. Wash the pellet with ice-cold 70% ethanol and dry it in a Speed-vac. Resuspend the DNA samples in ddH_2O at a concentration of 1-5 × 10^7 cell equivalents per μL (*see* Note 7). In yeast, HPV DNA is replicated at 1-5 copies per cell in the absence of E2 and 50 copies per cell in the presence of E2.

3.6. DNA Replication Assay of Plasmids Recovered From Yeast

In order to determine whether HPV target genomes are replicating episomally, a Southern analysis should be performed. In many cases, it is desirable to demonstrate that the HPV plasmids have undergone complete replication in yeast. In order to achieve this, the *Dpn*I resistance assay is utilized (1,13). In this assay, bacterially methylated DNA is digested by *Dpn*I and thus is discernable from DNA replicated in eukaryotic cells, which is unmethylated at *Dpn*I sites and therefore resistant to digestion (*see* Note 8).

3.6.1. Dpn I Resistance Assay

1. *Dpn*I digest approx 1 × 10^8 cell equivalents of DNA isolated from yeast for 24 h at 37°C. Include 2 ng of a control bacterially synthesized DNA as a means to monitor the completeness of *Dpn*I digestion.
2. Electrophorese DNAs on a 1% agarose gel along with copy number controls of the target plasmid, diluted to an appropriate concentration.
3. Transfer the DNAs to nitrocellulose using standard Southern blotting techniques (14).
4. Radiolabel the HPV DNA probe by use of a Rediprime kit, according to the manufacturer's instructions.
5. Probe the blot with the appropriate radiolabeled HPV DNA and wash the blot according to standard Southern techniques (14).
6. Visualize and quantify the DNA by use of a PhosphorImager.

The example demonstrated in Fig. 2B shows evidence that each of the plasmids (pPA103 and pPA106) is capable of low-copy episomal replication in yeast, as indicated by the presence of supercoiled and open-circle DNA forms.

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[7]An alternative to the yeast DNA miniprep method described in **Subheading 3.5.** is to use the Zymoprep yeast miniprep kit made by Zymo Research (Orange, CA). Simple instructions are provided with this kit, and we found it to be efficient for DNA recovery.

[8]When performing viral DNA replication experiments, as shown in Fig. 2B, particular attention should be paid to whether episomal forms of circular plasmids are observable. By comparing samples to appropriate control plasmids, super-coiled and open-circle forms of plasmids can be identified. An important analysis here is to digest the genomes with a convenient single-cutting enzyme to generate a linear fragment. If an appropriate-sized linear form is not observed, then there is a high likelihood that integration of the plasmid into the yeast chromosomes has occurred. As discussed in the methods section, a useful proof of DNA replication is given by resistance to digest of the DNA by *Dpn*I. Alternatively, *Mbo*I enzyme can be used in place of *Dpn*I. *Mbo*I digestion occurs only on DNA unmethylated at *Mbo*I sites, as is the case with DNA replicated in bacteria. *Mbo*I sites are protected when methylated during DNA replication in mammalian cells.

3.7. Modeling of HPV Trans-Acting Functions in Yeast

For many reasons, efficient expression of viral ORFs in yeast in *trans* to the replicating genomes becomes desirable. The example given in this chapter is of the effects of E2 expression on replication and transcription of full-length HPV-16 genomes in yeast. In keratinocytes, E2 functions in replication, maintenance, and transcription of the viral genome (15,16). In yeast, moderate E2 expression causes a 10-fold induction of genome copy number and a concomitant induction of viral E6-E7 mRNA expression (Fig. 3, left and right panels), which demonstrates the utility of this system. It is important to note that use of a relatively low expression-level promoter may be important to achieve the most biologically meaningful results (*see* **Notes** [9] and [10]).

3.7.1. mRNA Isolation From Yeast—Assessment of E2-dependent transcriptional effects necessitates use of a quantitative Northern analysis. A convenient method for isolation of total RNA from yeast is outlined as follows:

1. Resuspend pelleted yeast (from a 5-mL overnight culture) in 300 μL of buffer A.
2. Add 300 μL of phenol.
3. Mix thoroughly and incubate at 65°C for 5 min.
4. Vortex briefly.
5. Centrifuge the mixture for 2 min at 14,000*g* in a microfuge.
6. Recover supernatant and extract with chloroform/isoamyl alcohol (24:1).
7. Centrifuge the mixture for 2 min at 14,000*g* in a microfuge.
8. Precipitate the RNA by addition of 0.1 volume sodium acetate and 2.5 volumes of ethanol, followed by incubation at -20°C for 10 min.
9. To pellet the RNA, microfuge the tube at 14,000*g* for 10 min at 4°C.
10. Dry the pellet in a Speed-vac, resuspend the pellet in RNase-free water, and treat with RNase-free DNase I.

After isolation, approx 1×10^8 cell-equivalents of RNA should be analyzed by standard Northern techniques as prescribed in the *Current Protocols in Molecular Biology* manual (14). The described procedure for analysis of viral mRNAs is simple; thus, expressions of multiple transcripts are easily tested simultaneously.

3.8. HPV Pseudovirion Isolation From Yeast

The rationale for creation of the HPV/yeast system was to potentially use it as a means to create significant quantities of virus. Historically, papillomaviruses have proved difficult to propagate. The efficiency and rapidity of yeast culture methods allow recovery of approx 2.5×10^{10} yeast cells from a 250-mL culture grown in 24 h, which argues that production of infectious HPV in yeast could be made extremely efficient by use of this system. The virion

[9]Use of a galactose-inducible promoter to express L1 and L2 allows better control of protein stoichiometry during the virus assembly experiments. Our experience in the lab indicates that over-expression of certain *trans*-factors such as E1, and to a far lesser extent E2, can lead to recombination of plasmids, and therefore should be carefully controlled. It is also important to note here that large differences in copy number between expression and target HPV plasmids can lead to recombination between the plasmids, although it may not always occur. Careful control of protein expression and plasmid copy number is important to the overall stability of the system. Furthermore, inducible control of L1 and L2 helps to mitigate some of the potential safety issues with HPV psuedovirion production in yeast.

[10]The addition of E2 in the virion encapsidation strain is likely to be important for at least two reasons. First, E2 expression induces a copy number increase up to approx 50 copies per cell, which is likely to increase the efficiency of encapsidation. Second, E2 may enhance packaging according to bovine papillomavirus (BPV) studies (18) and is known to interact with L2, the minor capsid protein (19).

isolation protocol was created by modification of the procedure described by M. Ozbun (3), with changes specific for isolation of virus from yeast.

3.8.1. Pseudovirion Isolation Procedure

1. Grow the yeast strain harboring the HPV genome, E2, and the Gal-inducible L1 and L2 expression plasmids on a plate under nutritional selection in the presence of dextrose at 30°C for 2 d, so that an individual colony can be easily picked.
2. Inoculate a 5-mL culture from a single colony with selective medium in the presence of dextrose and grow overnight at 30°C with vigorous shaking.
3. Centrifuge the overnight culture at approx 2000*g* for 5 min. Wash the pellet in selective medium with galactose. Inoculate the washed cell pellet into a 25-mL culture with selective medium and galactose and allow it to grow at 30°C with vigorous shaking for 2 d. The cells should be above an OD_{600} of 1.4 (*see* Note 11).
4. Centrifuge the yeast pellet in 50-mL Falcon tubes by low-speed centrifugation (2000*g*).
5. Resuspend the pellet in 1 to 2 mL of solution 1, transfer to a 2-mL tube, wash, and re-pellet the yeast.
6. Resuspend the yeast in 1.5 mL of solution 1. Add 200 μL of 400-nm glass beads and vortex for 2 min, then chill on ice for 1 min. Repeat vortexing and chilling four times (*see* Note 12).
7. Centrifuge the mixture at 8000*g* in a microfuge for 10 min at 4°C. Transfer the supernatant to an SW41 centrifuge tube and fill the remaining volume of the tube up completely with solution 1.
8. Centrifuge the supernatants at 130,000*g* for 1 h in an SW41 rotor in an ultracentrifuge.
9. Pour off the supernatant (discard as biohazard) and resuspend the pellet in 50 μL of solution 2; scrape the bottom of the tube thoroughly and transfer to a microfuge tube.
10. Mix the material thoroughly and centrifuge at 8000*g* in a microfuge for 10 min at 4°C. Transfer the supernatant to a new tube. This is the clarified virus extract (*see* **Notes** [2] and [13–15]).

3.8.2. Analysis of Encapsidation by DNase Treatment—In order to determine the efficiency of HPV encapsidation, pseudovirus produced in yeast is subjected to DNase I sensitivity analysis. Pseudoviruses that are complete should protect internalized DNAs from DNase digestion. The protocol is as follows:

1. Incubate pseudovirus with 1 unit of DNase I in DNase buffer for 30 min at 37°C (1 unit of DNase I will digest 1 μg of DNA in 10 min at 37°C) in a convenient volume. Adjustment of the DNase I concentration may be necessary.

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[11] An OD_{600} reading at the point of harvest can be used to calculate the number of yeast cells (where OD_{600} of 1 is equal to approx 10^7 cells).

[12] Isolation of pseudovirions by means of vortexing yeast with glass beads is an efficient method. We noticed that it is important to take note of, and adhere to, vortexing conditions that yield the highest quality pseudovirion preparations. By electromagnetic analysis of different pseudovirus preparations, we noticed that there can be variability in the preparations in terms of the number of complete and broken pseudoviruses. An alternative to using a vortex for harvesting pseudovirus is to use a Beadbeater (Biospec, Bartlesville, OK). Again, optimization of the treatment should be performed to define the best conditions.

[13] Further physical-chemical analysis of HPV pseudovirions is important and can be achieved by performing a CsCl gradient (7) on the virus extract. This approach allows a determination of pseudovirus density shift due to the presence of encapsidated HPV DNA genomes.

[15] A step to allow maturation of virions isolated from yeast can be included by simply incubating the virions in buffer 2 at 37°C for 24 h.

2. Add an equal volume of DNase stop buffer.
3. Phenol/chloroform extract and precipitate the DNA.
4. Analyze DNase-treated and non-DNase-treated virion samples by Southern analysis and quantify the number of DNase-resistant DNA-containing units by comparison of DNA amounts with control DNAs (*see* Note 16).

An example of electron micrographs of HPV pseudovirions produced from yeast is shown in Fig. 4. In this example, L1 and L2 were induced in the presence or absence of full-length HPV 16 (pPA103). Note that only in the presence of full-length HPV 16 is the negative stain excluded from the centers of pseudovirions and obvious projections on the surface. These properties suggest that pseudovirions produced by the described yeast method may be identical to authentic virions (*see* Note [9] and [10]).

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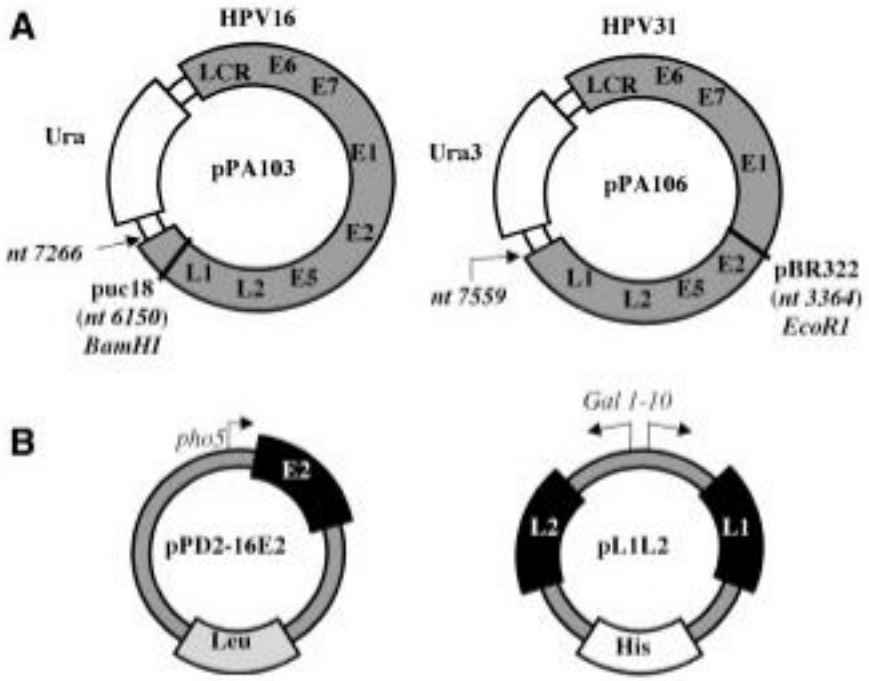

Fig. 1.

Human papillomavirus (HPV)/yeast plasmid constructs and HPV ORF expression vectors. Each HPV construct contains a *Ura3* nutritional marker placed in a convenient location in the genome. **(A)** The pPA103 vector, containing the HPV-16 genome, has the *Ura3* cassette inserted into nt 7266 of the genome, between the L1 ORF and the LCR. The puc18 backbone can be excised by a *Bam*HI digest. The pPA106 vector, which contains the HPV-31 genome, has the *Ura3* cassette inserted into an *Spe*I site at nt 7559 of the genome, at the 5′ end of the LCR. The pBR322 backbone can be excised by an *Eco*RI digest. **(B)** The pPD2-16E2 construct contains a *Leu2* selectable marker. The pho5 promoter drives expression of HPV-16 E2 at moderate levels when uninduced. The pL1L2 plasmid contains a bidirectional galactose-inducible promoter, which drives expression of HPV-16 L1 and L2.

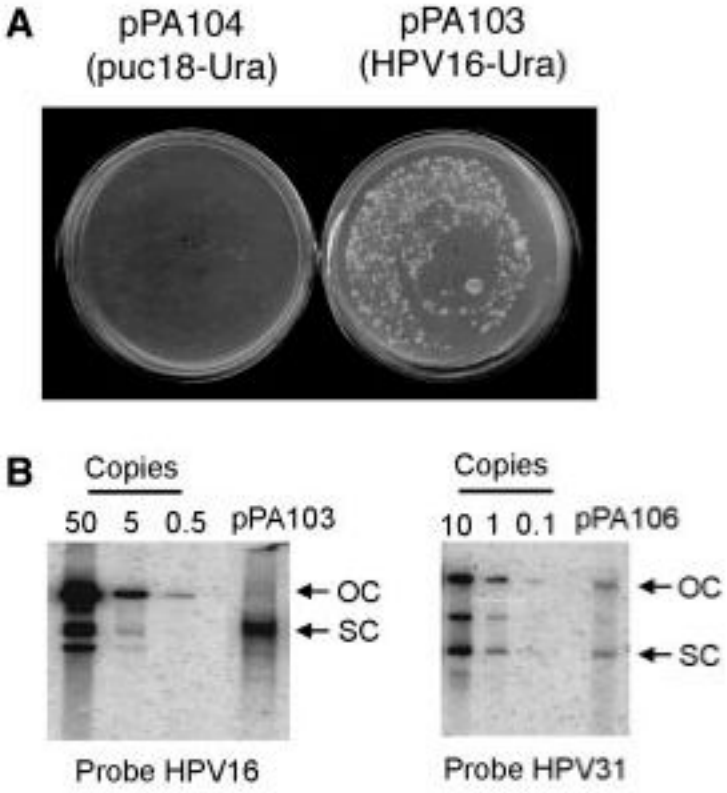

Fig. 2.

Example of colony formation and episomal replication of HPVs in yeast. **(A)** Two-hundred ng of plasmid DNA, either a control plasmid (pPA104; puc18-Ura) or pPA103 (HPV16-Ura), was transformed into haploid YPH500 yeast. Yeast were plated on minimal media lacking uracil. Plates were incubated at 30°C for 3 d prior to analysis and quantification of colony formation. **(B)** Colonies that were capable of growth in the absence of uracil were grown in 5 mL liquid culture overnight, and DNA was isolated. Approx 1×10^8 cell equivalents of DNA was loaded on to a 1% agarose gel and analyzed by Southern blot. Examples of episomal replication of pPA103 (HPV 16) and pPA106 (HPV 31) are shown on the left and right panels, respectively. The controls on the left of each blot represent the number of DNA copies per cell. OC = open-circular, SC = super-coiled.

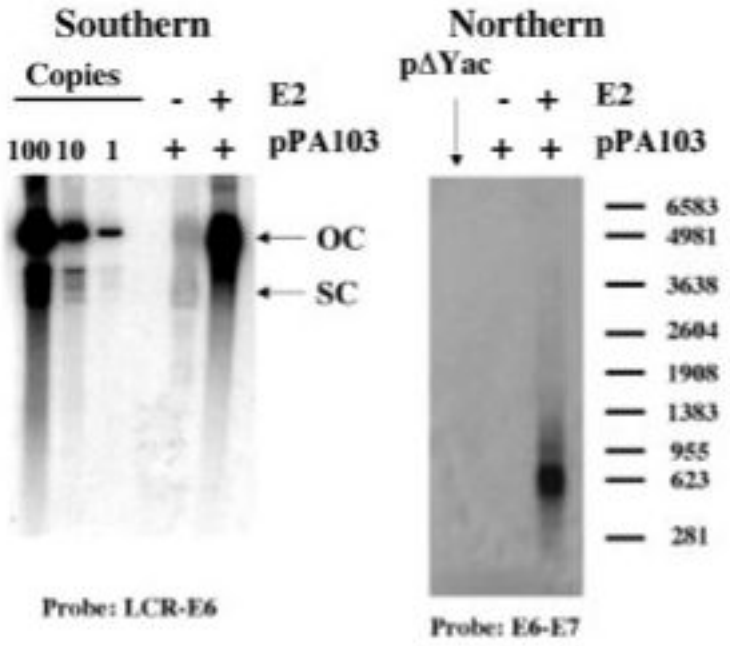

Fig. 3.

Trans-effects of E2 expression on the full-length HPV 16 in yeast; genome amplification and transcription from the viral genome. Yeast containing full-length HPV 16 (pPA103), either expressing HPV 16 E2 (pPD2-16E2) or not (pPD2), were subjected to Southern and Northern analysis to assay for E2-dependent DNA amplification and E6-E7 mRNA production, respectively. pΔYac is included as a negative control in these experiments. Probes for each of the blots are indicated below. The markers to the right of the Northern blot (right panel) represent the nucleotide lengths of RNA markers.

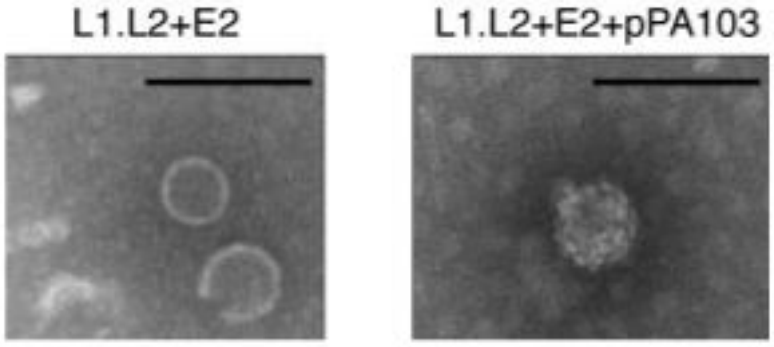

Fig. 4.

Virion assembly in yeast. Yeast containing pL1.L2 and pPD2-16E2 or pL1.L2 and pPD2-16E2 and pPA103 (lacking the puc18 vector) were induced to express L1 and L2 open reading frame an d allowed to grow for 1-2 d. Yeast were disrupted by vortex treatment with glass beads, and virus was recovered by differential centrifugation. Virus extracts were analyzed here by electron microscopy using the phospho-tungstenate staining method. Each bar represents 100 nm.