Isolation and quantification of MmuPV1 virions from papillomas

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Abstract

In this section we describe our method of isolation and quantification of MmuPV1 virions from papillomas arising in nude mice that can be serially passaged or used to infect other strains of mice. In our studies we have used both crude virus extracts or purified virus particles. Crude virus extract refers to virus present in supernatants obtained by clarifying papillomatous tissue extracts from nude mice. Our method of generation of crude virus extract has been modified from a previous protocol used to isolate merkel cell polyomavirus from human skin swabs(1). Purified virus particles refer to crude extract clarified over an optiprep gradient. Optiprep gradients have been used to purify papillomavirus generated in *vitro* (2) and have been described in detail on Dr. Chris Buck's website: https://home.ccr.cancer.gov/LCO/pseudovirusproduction.htm#_ENREF_7. We measure the amount of encapsidated viral genomic DNA to determine the concentration of "viral genome equivalents" (VGE) in any given stock of virus. For this we use a standard agarose gel electrophoresis followed by SYBR-green staining. The same gel can also be used for southern analysis using MmuPV1-specific probes (3) by nick translation as we have described in detail previously(4). Since virus yields from papillomas of nude mice are very high, we have found agarose gel electrophoresis to be sensitive enough to accurately determine virus concentrations. Other methods of virus quantification have also been described in the literature(5, 6).

References:

1. Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL, Buck CB. 2010. Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. Cell host & microbe **7:**509-515.

2. **Buck CB, Thompson CD.** 2007. Production of papillomavirus-based gene transfer vectors. Current protocols in cell biology **Chapter 26:**Unit 26 21.
3. **Uberoi A, Yoshida S, Frazer IH, Pitot HC, Lambert PF.** 2016. Role of Ultraviolet Radiation in Papillomavirus-Induced Disease. PLoS pathogens **12:**e1005664.
4. **Lorenz LD, Rivera Cardona J, Lambert PF.** 2013. Inactivation of p53 rescues the maintenance of high risk HPV DNA genomes deficient in expression of E6. PLoS pathogens **9:**e1003717.
5. **Cladel NM, Budgeon LR, Cooper TK, Balogh KK, Hu J, Christensen ND.** 2013. Secondary infections, expanded tissue tropism, and evidence for malignant potential in immunocompromised mice infected with Mus musculus papillomavirus 1 DNA and virus. Journal of virology **87:**9391-9395.
6. **Handisurya A, Day PM, Thompson CD, Buck CB, Pang YY, Lowy DR, Schiller JT.** 2013. Characterization of Mus musculus papillomavirus 1 infection in situ reveals an unusual pattern of late gene expression and capsid protein localization. Journal of virology **87:**13214-13225.

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Materials

Homogenizer PowerGen 125 by Fisher Scientific

Swinging bucket rotor Type 55 Ti SW 55 Ti by Beckman Coulter

Protocol

Harvesting of virus particles from papillomas

Step 1.

1. Euthanize the animal from which tissue has to be harvested by CO_2 exposure as per IACUC guidelines.
2. Use a scalpel to excise the papilloma from the ear taking care to not collect the surrounding epithelia (non-papilloma tissue).

- Weigh the papilloma and place in a 15ml round bottom falcon tube. For transportation tube is kept on ice till ready for processing at lab bench

1. For every 10mg wart 700µl DPBS is added to the tube
2. Papilloma is homogenized for less than one minute using homogenizer and suspension is immediately transferred to a siliconized 1.5ml sterile eppendorf tube.
3. In a 50ml falcon tube add about 25ml DPBS and wash homogenizer to collect any debris that might have got stuck in the homogenizer blades. Centrifuge falcon tube to collect pellet and aspirate the supernatant. Resuspend pellet in 42.5µl DPBS and combine with above fraction.

- Add 7.5µl of Triton-X-100 solution (1% Triton-X-100).
- Add 2µl Benzonase endonuclease and incubate at 37°C for 20 minutes inverting tube every 5 minutes.

1. Add 2-3mg of Collagenase H and vortex sample and incubate at 4°C
2. Adjust NaCl concentration of suspension to 0.8M by adding appropriate amount of 5M NaCl (Since suspension is 750µl, add 127.5µl of 5M NaCl).
3. Centrifuge tube for 5 mins at 5000 x g. Transfer supernatant to fresh siliconized tube. Collect a 20µl aliquot of the virus in a separate tube for quantification.

At this point you can proceed to use crude virus extract for serial infections or purify over an optiprep gradient. Virus extract can be stored at -80°C until future use.

Purification of virus extract over optiprep gradient

Step 2.

1. At this point you can proceed to use crude virus extract for serial infections or purify over an optiprep gradient.
2. At least 2 hours before spinning, prepare a three-step optiprep gradient (27%-33%-39%) and let

it rest. First pipette 1.5ml of 27% optiprep solution directly to the 5ml centrifuge tube. Now using a long bone-marrow biopsy long needle, dispense 1.5ml 33% optiprep solution slowly at the bottom of the tube. If layered correctly you will see three separate layers of the two solutions. Now dispense 1.5ml of 39% optiprep solution at the bottom of the tube that will result in three steps of the gradient. Let the gradient stand at room temperature for 2-4 hours.

- Gently layer crude lysate on top of optiprep gradient and spin at 16°C, at 50000 RPM for 3.5 hours using the SW-55 swinging bucket rotor. Prior to loading the tubes balance the tubes using DPBS by weighing using a gram scale.

1. After centrifugation carefully remove the 5ml tube using forceps and mount on a clamp stand. With the help of a 27-gauge needle carefully make a hole at the bottom of the tube slightly off center. Now collect virus fractions in a drop-wise manner as follows: collect fractions 1 and 2 as approximately 500µl, fractions 3-10 as 250µl each, fractions 11-14 as 500µl each.
2. Also remove 20µl aliquots from each fraction in separate eppendorf tubes for quantification.
3. Store the fractions and their respective aliquots at -80°C until further analysis or use.

Determination of virus concentration using agarose gel electrophoresis

Step 3.

1. Virus concentration can be determined by calculating viral genome equivalence over a 0.6% agarose gel in 1X TAE followed by staining with SYBR green.
2. Release viral DNA by denaturing the viral capsid by adding 10µl virus lysis buffer (0.1% Proteinase K, 0.5% SDS, 25mM EDTA in ddH_2O) to 10µl virus aliquot and incubate at 56°C for thirty minutes.

- Load entire volume and resolve via gel electrophoresis running known DNA concentration standards in parallel.

Warnings

MmuPV1 infects laboratory mice. As such please treat any material harvested from mice carefully to prevent contamination of mouse vivaria. Therefore we recommend that readers familiarize themeselves with the following publications to take necessary precautions to decontaminate areas with MmuPV1:

1. Meyers J, Ryndock E, Conway MJ, Meyers C, Robison R. Susceptibility of high-risk human papillomavirus type 16 to clinical disinfectants. *Journal of Antimicrobial Chemotherapy*. 2014;69(6):1546-1550. doi:10.1093/jac/dku006.

2. Ryndock E, Robison R, Meyers C. Susceptibility of HPV16 and 18 to high level disinfectants indicated for semi-critical ultrasound probes. *Journal of Medical Virology*. 2016;88(6):1076-1080. doi:10.1002/jmv.24421.

We recommend decontaminating areas and tools with 5% bleach followed by rinsing with water followed by wiping with 70% ethanol.