Supplementary Information:

A Human Papillomavirus Whole Genome Plasmid Repository: A Resource for HPV DNA Quality Control Reagents

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Materials and Methods

HPV DNA templates for construction of CDC HPV Plasmids

Plasmids for HPV6, HPV45 and HPV51 were received from the International HPV Reference Center hosted by German Cancer Research Center (Germany). Plasmids for HPV31, HPV35, HPV56, HPV58 and HPV59 were received from the International HPV Reference Center hosted by the Karolinska Institute (Sweden). Plasmids for HPV33, HPV39, HPV66 and HPV68 were received from the Pasteur Institute (France). Plasmids for HPV11, HPV16, HPV18 and HPV52 were received from American Type Culture Collection (USA). Non-CDC HPV plasmids were propagated by transformation into *E.coli* cells, plasmids were purified using QIAprep Spin Miniprep Kit (QIAGEN, Germany), and HPV insert sequences in plasmids were verified by restriction enzyme digestion followed by Sanger sequencing with HPV type specific primers. Small DNA fragments for HPV45 (nucleotides from 1-500 bp), HPV51 (nucleotides from 1-378 bp) and HPV59 (nucleotides from 1-545 bp) were synthesized and were directly used as template in PCR reactions. The synthetic DNA fragment of HPV51 was made to replace the missing 232 bp sequence from the non-CDC HPV51/pUC13 plasmid, and the synthetic fragments for HPV45 and 59 were made so that the PCR amplicon to be assembled with other DNA fragments was larger than 300 bp. All synthetic fragments were synthesized by Twist Biosciences (USA).

Construction of CDC HPV whole genome plasmids

Maps of the HPV whole genome plasmids with DNA fragments to be assembled were first generated in silico using SnapGene software. The new HPV plasmids were designed so that none of the HPV open reading frames are interrupted. The vector backbone of pGEMT Easy-01 plasmid was split into two DNA fragments, vector-bkb-1 (67-2045 bp) and vector-bkb-2 (68-2073 bp), at a region that encodes the ampicillin resistance gene so that the assembly of two

vector backbone fragments detected through antibiotic selection of bacterial colonies could be used as a proxy for successful assembly of DNA fragments. Primers for amplification of HPV DNA fragments were designed to contain an overlapping sequence (20-50 bp) between DNA fragments to be assembled (Table S1). DNA fragments for HPV and vector backbones with overlapping ends were synthesized using PCR. HPV fragments were generated using non-CDC HPV plasmids or synthetic HPV fragments as a template, and pGEMT Easy-01 plasmid was used as a template to generate vector-bkb-1 and vector-bkb-2 fragments. PCR reactions were conducted in a total volume of 20 µL and contained 10 ng of template, 0.5 µM primers and 10 µL of 2x PrimeSTAR Max DNA Polymerase master mix (Takara Bio, USA). PCR reactions were carried in Proflex thermocycler (ThemoFisher Scientific, USA) and typical cyclic conditions were initial denaturation at 98°C for 2 min, 33 cycles of 98°C for 15 sec, 55°C for 15 sec, extension at 72°C (10 sec/kb), and final extension at 72°C for 5 min. Primers for creating overlapping DNA fragments required optimization of annealing temperatures as some DNA fragments are longer than standard primers and the range of GC content and T_m among primers was wide.

PCR amplicons of desired length were purified from agarose gel using the Zymoclean Gel DNA recovery kit (Zymo Research, USA) following the manufacturer's instructions. Overlapping DNA fragments were assembled in a 20 μ L reaction volume containing 0.025 pmol of vector-bkb-1, 0.025 pmol of vector-bkb-2, 0.05 pmol of each HPV DNA fragment and 10 μ L of 2x NEBuilder HiFi DNA assembly master mix. The assembly reaction was carried out by incubation at 50°C for 60 min followed by transformation of 0.5 to 2 μ L of reaction products into Mix and Go DH5 α competent cells or Max Efficiency Stbl2 cells (ThermoFisher Scientific, USA) following the manufacturer's recommendation. Bacterial colonies were screened with

colony PCR using vector specific primers (pGEMT-F and pGEMT-R) to identify clones containing empty vector and HPV type specific primers to screen for HPV positive colonies (Table S1). DNA from bacterial colonies were prepared by resuspending individual colonies in a lysis buffer (10 mM Tris-HCl pH 8.0, 0.1% Triton X-100) followed by incubation at 95°C for 10 min. Colony PCR reactions were conducted in a total volume of 10 μ L and contained 1 μ L of bacterial lysate as template, 0.5 µM primers and 10 µL of 2x GoTaq Green master mix. Colony PCR conditions include initial denaturation at 95°C for 5 min, 25 cycles of 95°C for 30 sec, 55°C for 30 sec, extension at 72°C for 30 sec, and final extension at 72°C for 10 min. Bacterial clones showing PCR amplicons with only HPV type specific primers were selected. Plasmid DNA was purified using QIAprep Spin Miniprep, verified by restriction enzyme digestion and confirmed by Sanger sequencing following standard protocols. Large scale plasmid purification of all HPV whole genome plasmids was done using ZymoPure II Plasmid Maxiprep Kit (ZymoResearch, USA) following the manufacturer's protocol. HPV plasmids were diluted in nuclease-free DNA suspension buffer (10 mM Tris-HCl pH 8.0, 10 mM EDTA; Teknova Inc, USA). All plasmid preparations had A_{260}/A_{280} and A_{260}/A_{230} values greater than 1.8 as assessed with NanoDrop, and the HPV sequence from large preparations was verified by Sanger sequencing. Plasmid concentrations were determined using Qubit Fluorometer (ThermoScientific, USA).

DNA	Forward Primer	*D-1	DNA
Fragment	Reverse Primer	Primer sequence (5-3)	Template
Vector-bkb-1	1-F-AmpR	CCGCTGTTGAGATCCAGTTCGATGTAACC	
(1040 bp)	1-R-pGEMT	GTGATTAATCGAATTCCCGCGGCCG	pGEMT Easy-01
Vector-bkb-2	2-F-pGEMT	TAGTGAATTCGCGGCCGCCTG	DGEMT Easy-01
(2006 bp)	2-R-AmpR	GGTTACATCGAACTGGATCTCAACAGCGG	poemi easy-or
Vector fragment	pGEMT-F	GTAACGCCAGGGTTTTCCCAG	DNA f rom
(258 bp)	pGEMT-R	CAGCTATGACCATGATTACGCCAAG	bacterial clones
HPV6-F1	HPV6-F1-F	CCGCGGGAATTCGATTAATCACGTTAATAACAATCTTGGTTTAAAAAATAGGAGGGACC	
(4763 bp)	HPV6-F1-R	AGACAC A AT AG A TGG A TC C G AAGG GGC C AC AGGC TC C AC CACCACAG	HPV6/pUC19
HPV6-F2	HPV6-F2-F	GTGGAGCCTGTGGGCCCCTTCGGATCCATCTATTGTGTCTTTAATTGAAGAATCGG	
(3316 bp)	HPV6-F2-R	CAGGCGGCCGCGAATTCACTATATAAGAAGGAAATATGTAGGGTGTGGATAACCG	
HPV6 fragment	HPV6-4525-44-F	GTGGAGCACAACACCATTGC	DNA f rom
(318 bp)	HPV6-4820-42-R	TGCAGGGGTAGTTGTTTCAGAGG	bacterial clones
HPV11-F1	HPV11-F1-F	CGGCCGCGGGAATTCG ATTAATC ACCTTA ATAACAATCTTAGTTTAAAAAAAGAGGAGG	HPV11/pBR322
(7117 bp)	HPV11-F1-R	CTCATATCCTTATAGGGATCCTGTTTTTCTTTTCAGGTGTGGGTTTCTG	
HPV11-F2	HPV11-F2-F	CACACCTGAAAAAAGAAAAACAGGATCCCTATAAGGATATGAGTTTTTGGGAGGT	HP\/11/pBR 322
(904 bp)	HPV11-F2-R	CAGGCGGCCGCGAATTC AC TA TATA AGAA GGAA ATATGTA GGG TG TGG GTAACC	
HPV11 fragment	HPV11-6842-63-F	GAATACATGCGCCATGTGGAGG	DNA f rom
(415 bp)	HPV11-7234-56-R	GTTTTCGTTTGGGGGGCTGTAGAG	bacterial clones
HPV16-F1	HPV16-F1-F	CGGCCGCGGGAATTCG ATTAATC ACAC TACAA TAATTCATGTATAAAAC TAAGGGCGTA	HPV16/
(6199 bp)	HPV16-F1-R	GCAACATTGGTACATGGGGATCCTTTGCCCCAGTGTTCCCCTATAG	pBluescript SK-
HPV16-F2	HPV16-F2-F	TAGGGGAAC AC TGGGGC AA AGG A TCCCC AT GT ACC AA TG TT GC AG	HPV16/
(1796 bp)	HPV16-F2-R	CAGGCGGCCGCGAATTC AC TA TTA GTA TTA TTA TA TA AG TTGCTTG TA A ATGTG TA AC	pBluescript SK-
HPV16 fragment	HPV16-6036-57-F	GTGCTTATGCAGCAAATGCAGG	DNA f rom
(337 bp)	HPV16-6350-72-R	CTGTCGCCATATGGTTCTGACAC	bacterial clones
HPV18-F1	HPV18-F1-F	GCGGGAATTCG ATTAATC AC ATTAA TAC TTTTAACA ATTG TAG TA TA TAA AAAA GGGA G	HPV18/pBR322
(2471 bp)	HPV18-F1-R	CTAGTGGAATTC AC A AA TG AT ATT AC TGC TCCTT GT AT AAAG TG	
HPV18-F2	HPV18-F2-F	GCAGTAATATCATTGTGAATTCCACTAGTCATTTTTGGTTGG	HPV18/pBR322
(5456 bp)	HPV18-F2-R	CAGGCGGCCGCGAATTC AC TA GAAAAG TA TAG TA TG TGCTGCCCAACC	
HPV18 fragment	HPV18-2260-82-F	AGTGCAATTCCTGCGATACCAAC	DNA f rom
(405 bp)	HPV18-2639-64-R	GGCCATCTATTATCCTTTGCTGGATG	bacterial clones
HPV31-F1	HPV31-F1-F	GCGGGAATTCGATTAATCACTAATAATAATAATCTTAGTATAAAAAAGTAGGGAGTGACCGAAAGTG	HPV31/pUC19
(3394 bp)	HPV31-F1-R	AGGTTTTG G AAT TCG ATG TGG TG GT GT GT TGGC TG TT GG TAGC TT TG TAAC	
HPV31-F2	HPV31-F2-F	AGCCAAC AAC ACC ACC AC ATCG AA TTCC AA AACCTGCGCCTTGGGCACCAGTG	HPV31/pUC19
(2298 bp)	HPV31-F2-R	GTTACATATTC ATCCGTGCTTAC AACTTTAG AC ACTGGGACAGG TGG TAAGTAGAC	
HPV31-F3	HPV31-F3-F	AGTGTCTAAAGTTGTAAGCACGGATGAATATGTAACACGAACCAACATATATTATC	HPV31/pUC19

Table S1 List of primers used for construction of CDC HPV whole genome plasmids

(2325 bp)	HPV31-F3-R	CTGCAGGCGGCCGCGAATTCACTAAAGTATAAAAAGAACAATTGCTTGTAAAACTGTAAC	
HPV31 fragment	HPV31-3116-40-F	GATGGCCAATGTACTGTTGTGGAAG	DNA f rom
(428 bp)	HPV31-3522-43-R	GTTTGGTTTGTGCATGCAGCTG	bacterial clones
HPV33-F1	HPV33-F1-F	CGGCCGCGGGAATTCG A T T AA TC AC G TAA AC TA TA A TGCCAA G T T TAAAA AAG TAG GG TG TAAC	HPV33/
(2839 bp)	HPV33-F1-R	ATCAGCTTCGTAAAGATCTAGTATTTTCTCCTGCACTGCATTTAAACGTG	pLink322
		ATGC AGTGC AGG AG A A A A T ACT AG A TCTTT ACG A AGC TG AT AAAAAC TGA T TTACCA TC	
HPV33-F2	HPV33-F2-F	CAGGCGGCCGCGAATTC AC TATATTA TA TAA AAGA TTA TAAA TGACCAA TA TG ACCTAAA ACGG TTAG	HPV33/
(5157 bp)	HPV33-F2-R	тс	pLink322
HPV33 fragment	HPV33-2640-66-F	GATGAAAATGGTAACCCAGTGTATGCA	DNA f rom
(392 bp)	HPV33-3003-31-R	GCAATGTCCATTGGCTTG TAC TA TACTG	bacterial clones
HPV35-F1	HPV35-F1-F	CGGCCGCGGGAATTCG A T T AA TC AC CCCTA TAA AAAAAAACAGGG AG TGACCGAA AAC	HPV35/pBR322
(1007 bp)	HPV35-F1-R	CATTITIC GTCCTC TG AC AC TGG A TC CCCCGT AC G TC T ACT A CT ACT GC TTC	Plasmid 2
HPV35-F2	HPV35-F2-F	GCAGTAGTTAGTAGACGTACGGGGGGATCCAGTGTCAGAGGACGAAAATGAAG	HPV35/pBR322
(4130 bp)	HPV35-F2-R	CTATAATG TCC AT A AAG TC AGG A TC CGG AGC T A AGC T A A T A TC C TCA TGC TC	Plasmid 1
HPV35-F3	HPV35-F3-F	GATATTAGCTTAGCTCCGGATCCTGACTTTATGGACATTATAGCTTTACATAG	HPV35/pBR322
(2880 bp)	HPV35-F3-R	CAGGCGGCCGCGAATTC AC TAATAATTGTTAC TAAC TA	Plasmid 2
HPV35 fragment	HPV35-751-73-F	GCGACACTACGTCTGTGTGTACA	DNA f rom
(411 bp)	HPV35-1139-61-R	CACGCTGCTAAGTGGACTACTAG	bacterial clones
HPV39-F1	HPV39-F1-F	GCGGGAATTCG ATTAATC ACCTTATAACATTTTATAAGTATCTTGTTTAAAAAAAAGGGA	HPV39/pSP65
(6862 bp)	HPV39-F1-R	GTCCAATATAGAGGAATTCATAGTGTGAATATAAGACATAACATCAGTTGTTAATG	
HPV39-F2	HPV39-F2-F	GTCTTATATTC AC ACTATG AATTCCTCTATATTG G AC AATTGG AA TTTTGCTGTAG	HPV39/pSP65
(1049 bp)	HPV39-F2-R	CAGGCGGCCGCGAATTC AC TA TAAAAGTA TA GG TA TGTA TG	
HPV39 fragment	HPV39-6912-37-F	GCATCCTTTTGACATGTAATGGCTGC	DNA from
(246 bp)	HPV39-6692-19-R	CATACCTTCTACATATGATCCTTCTAAG	bacterial clones
HPV45-F1	HPV45-F1-F	GCCGCGGGAATTCG A TTAATC AC AA TAC T TT TAACAATTA TAC TAC	HPV45 synthetic
(546 bp)	HPV45-F1-R	CATGTATTAC ACTGCCCTCGG T ACT GTCC AGCT A TGCT GTG AA A TC TTC	fragment
HPV45-F2	HPV45-F2-F	GATTTC AC AGC A TAGC TG G AC AG TACC G AGG GC AGTG TA A TAC A TG TTG	
(3678 bp)	HPV45-F2-R	TAC ACTGTAC TG TAC ATTAT AGTA ATTA TTGTA TGG TG TA A AGCA TGCATATG	
HPV45-F3	HPV45-F3-F	ACCATAC A ATA ATT ACT A TA ATG TAC AGT AC AGT GT A ACA TACCTGTGATGTG	HPV45/ pGEM4
(3761 bp)	HPV45-F3-R	CAGGCGGCCGCGAATTC AC TAAAGAAAAGGTATGTGTTATAGGGCCCAAC	
HPV45 fragment	HPV45-3939-61-F	TCTGTGTGCCTTTATGTGTGCTG	DNA f rom
(377 bp)	HPV45-4291-15-R	CACGTACCGGATTGCTTACATGTTC	bacterial clones
HPV51-F1	HPV51-F1-F	GCCGCGGGAATTCG ATTAATC AC AACAATTATCTTG TA AAAAC TAGG GT GT AACCG	HPV51 synthetic
(420 bp)	HPV51-F1-R	TCGATAAATCATATAAGCTTTTTTTAGTAATTGCCTCTAATGTAGTACCATACACAG	fragment
HPV51-F2	HPV51-F2-F	TAGAGGC AATTAC TAAAAAAAGC TTATATG ATTTATCG ATAAGG TG TCA TAGA TG TC	HPV51/ pUC13
(3888 bp)	HPV51-F2-R	GTACCTTCAACCTTATTCACAACATCAGGAGGACATGTACCAGCAGCTTTG	
HPV51-F3	HPV51-F3-F	CTGGTACATGTCCTCCTGATGTTGTGAATAAGGTTGAAGGTACTACATTGGC	HPV51/ pUC13
(3629 bp)	HPV51-F3-R	CAGGCCGCCGCGAATTC AC TAAAAATGTTATAAGAAAAAATGTTGCAAGCATAGGC	
HPV51 fragment	HPV51-163-89-F	ATGCACAATATACAGGTAG TG TG TG TG	DNA f rom

(358 bp)	HPV51-501-20-R	GTTGCCAGCAATTAGCGCAT	bacterial clones
		GCCGCGGGAATTCG A TT A A TC AC TAA A TT A TAA TC TT A TAC TAG TAA AAAA TAG GG TG TAACCGAA AA	
HPV52-F1	HPV52-F1-F	CG	HPV52/pUC19
(3395 bp)	HPV52-F1-R	AGC AGTTTC AGT AG TG G AT ACT TC G TT AC T AG AT AC AG A TG CAG GACAAAC	
HPV52-F2	HPV52-F2-F	CATCTGTATCTAGTAACGAAGTATCCACTACTGAAACTGCTGTCCAC	
(4235 bp)	HPV52-F2-R	GACCAACCGAATTCGGTTAGGATTTAAAATGGTGGATAGTACAAAATG	HPV52/ pUC19
HPV52-F3	HPV52-F3-F	ATCCACCATTTTAAATCCTAACCGAATTCGGTTGGTCTTGGCACAACTTTG	
(429 bp)	HPV52-F3-R	CAGGCGGCCGCGAATTCACTATAAATTATAAAAAAAAAGTGGTTGTGGGTACGGTAAC	HPV52/ pUC19
HPV52 fragment	HPV52-3159-87-F	ACTTGGTGAGTGTGAATGTACAATTGTAG	DNA from
(337 hn)	HPV52-3469-95-R	GTACTIGGIGTLICI GGAG IC IG IGAC	bacterial clones
			bacterial ciones
HF V30-F 1			HPV56/pT713
(5555 bp)	HPV56-F1-R	CAAAGGAGGATCCCTGTATATATACATCATGGGTAACATCATAAGGAGAC	
HPV56-F2	HPV56-F2-F	AIGAIG TATATATAC AGGG A TCC TCC TTTGCATTATGGCC TG TG TATTTTTTAG	HPV56/pT713
(2362 bp)	HPV56-F2-R	CAGGCGGCCGCGAATTC AC TAAACAATTAAAAGAAACCTGTTTTTGCACGAC	
HPV56 fragment	HPV56-5304-27-F	TGTCTAGCCAGTCAGTTGC TACAC	DNA f rom
(355 bp)	HPV56-5634-58-R	CCTTTGAAACAGGTGTTGGAGGTAG	bacterial clones
HPV58-F1	HPV58-F1-F	CCGCGGGAATTCG ATTAATC ACCTAAACTATAATGCCAAA TCTTG TAAAAAACTAGGG TG	HPV58/pLink
(7053 bp)	HPV58-F1-R	CAAAG G AA ACTG A TCT AG A TCT GC AG A A AAC TTT TCCT TT A AG T TAACCTCC	
HPV58-F2	HPV58-F2-F	ACTTAAAGGAAAAGTTTTCTGCAGATCTAGATCAGTTTCCTTTGGGACGAAAG	
(858 bp)	HPV58-F2-R	CAGGCGGCCGCGAATTC AC TATATTATATAAAATGTTGAAACATGAACAATGTGACCCA	HP V50/PLINK
HPV58 fragment	HPV58-6868-89-F	CGTCTGCCAGTTTACAGGACAC	DNA f rom
(232 bp)	HPV58-7077-99-R	GTAGTAGGGGCCGAACGTTTTAG	bacterial clones
HPV59-F1	HPV59-F1-F	CGGCCGCGGGAATTCG ATTAATC ACGTTAAGACCGAAAACGGTGCATA TA AAGG TAG	HPV59 synthetic
(588 bp)	HPV59-F1-R	GTGTTGCTTTTGGTCC ATGC ATTG TTTT AC ACC AGTGT TTCACTACG	fragment
HPV59-F2	HPV59-F2-F	ACACTGGTGTA AA AC AATGC ATGGACC AAA AGC AAC AC TTTGTGAC	
(4012 bp)	HPV59-F2-R	TAGATG G ATC TG T AGG TC C AAC AG GTTC AA T A AC T AC T	неузэ/росэ
HPV59-F3	HPV59-F3-F	CAGTAGTTATTGAACCTGTTGGACCTACAGATCCATCTATAGTTACATTAG	
(3420 bp)	HPV59-F3-R	CAGGCGGCCGCGAATTC AC TACGTTTTCGGTTACACCCTTTTTTATTACTATGA	HFV59/p0C9
HPV59 fragment	HPV59-4360-82-F	ATATTGCAGTGGACCAGCCTAGG	DNA f rom
(369 bp)	HPV59-4705-28-R	GACAGAAGGGTCTGTAAATGCAGG	bacterial clones
		CGGCCGCGGGAATTCGATTAATCACGAAAGTTTCAATCATACTTATTATATTGGGAGTAACCGAAA	
		TGGGTTTAGGAC	HPV66/pBR322
(3581 bp)	HPV66-F1-R	ACTTCTAC TGTTGGCGTTGTTACTGATGTCTGTGTGTGTCTGTTGTGACACAGTGTGCGTAG	
HPV66-F2	HPV66-F2-F	CACAAC AG AC AC AG AC ATC AG TA AC A AC	
(3989 bp)	HPV66-F2-R	AGGCTAGGC AACCG AATTCGG TTGC ATGC ATAA AATG GCGTAC	HPV66/pBR322
HPV66-F3	HPV66-F3-F	CATTITATGC ATGC AACCG AA TICG GT TGCC TAGCC TITIGIC	
(380 bp)	HPV66-F3-R	CAGGCGGCCGCGAATTC AC TAAACAATTAAAAGAAACCTGTTTTAGCACGACC	HPV66/pBR322
HPV66 fragment	HPV66-3312-36-F	GTCCTGACTCTGTGTCTAGTACCTG	DNA f rom
(462 bp)	HPV66-3752-73-R	AGGTGTCCCGTTGTGTTTCATC	bacterial clones
1	1		1

HPV68-F1	HPV68-F1-F	CGGCCGCGGGAATTCG ATTAATC ACATGGCGC TATTTCACAACCCTGAGGAAC	HPV68/
(719 bp)	HPV68-F1-R	TACAACACAGACACTGAATTCTGTGACGCTGTTGTTCGTCCCGTC	pBluescript SK-
HPV68-F2	HPV68-F2-F	ACGGGACG AAC AAC AGC GTC AC AG AA TTC AG TG TC TG TG TG TAACAA GG	HPV68/
(3562 bp)	HPV68-F2-R	AGTACCTTC AACCTTATTTATAAC ATC AG AAGG AC ATG TCCCTG ATTGTTTAC	pBluescript SK-
HPV68-F3	HPV68-F3-F	CAATCAGGGACATGTCCTTCTGATGTTATAAATAAGGTTGAAGGTACTACACTTGCAG	HPV68/
(3680 bp)	HPV68-F3-R	CAGGCGGCCGCGAATTC AC TA TAGTATAGAGAACTGCTGTGTTCAGCTTTATATAC	pBluescript SK-
HPV68 fragment	HPV68-448-72-F	AGACGCATACGTCAAGAAACACAAG	DNA f rom
(366 bp)	HPV68-792-13-R	CTGGGTTTCAGTTGCACACCAC	bacterial clones

[#]Overlapping sequence in each primer is highlighted in bold and is used for creating overlapping

DNA fragments.



Figure S1. Map of plasmid (HPV16/pBR22) used for generating HPV16 International Standard. The plasmid contains full-length HPV16 genome which is cloned into pBR322 backbone using unique BamHI site. The L1 gene is split into two regions of the vector backbone.



Figure S2. (**A**) Map of pGEMT Easy-01 plasmid. The plasmid is used as vector backbone to construct CDC HPV whole genome plasmids. (**B**) Multiple cloning site of pGEMT Easy-01 plasmid is shown. The plasmid is a derivative of pGEMT Easy vector and was made by addition of two nucleotides, TA (highlighted in red). The HPV genomes of different HPV types are cloned between C67 and T68 nucleotides and position is shown by arrow.



Figure S3. (A) Map of non-CDC HPV6 (HPV6/pUC19) plasmid. The L2 gene is split into two regions of the vector backbone. (B) Map of CDC HPV6 (HPV6/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S4. (**A**) Map of non-CDC HPV11 (HPV11/pBR322) plasmid. The L1 gene is split into two regions of the vector backbone. (**B**) Map of CDC HPV11 (HPV11/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S5. (**A**) Map of non-CDC HPV16 (HPV16/pBluescript SK-) plasmid. The L1 gene is split into two regions of the vector backbone. (**B**) Map of CDC HPV16 (HPV16/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S6. (**A**) Map of non-CDC HPV18 (HPV18/pBR322) plasmid. The E1 gene is split into two regions of the vector backbone. (**B**) Map of CDC HPV18 (HPV18/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S7. (**A**) Map of non-CDC HPV33 (HPV33/pLink322) plasmid. The E1 and E2 genes are split into two regions of the vector backbone. (**B**) Map of CDC HPV33 (HPV33/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S8. (A) Map of partial genome non-CDC HPV35 (HPV35/pBR322) plasmid 1. The plasmid contains partial sequence of E1 and L2 genes. (B) Map of partial genome non-CDC HPV35 (HPV35/pBR322) plasmid 2. The plasmid contains partial sequence of E1 and L2 genes.
(B) Map of CDC HPV35 (HPV35/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S9. (A) Map of non-CDC HPV39 (HPV39/pSP65) plasmid. The L1 gene is split into two regions of the vector backbone. (B) Map of CDC HPV39 (HPV39/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S10. (A) Map of non-CDC HPV45 (HPV45/pGEM4) plasmid. (B) Map of CDC HPV45 (HPV45/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S11. (**A**) Map of partial genome non-CDC HPV51 (HPV51/pUC13) plasmid. The E6 gene is split into two regions of the vector backbone and is missing 232 bp of sequence from its open reading frame. (**B**) Map of CDC HPV51 (HPV51/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S12. (A) Map of non-CDC HPV52 (HPV52/pUC19) plasmid. (B) Map of CDC HPV52 (HPV52/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S13. (**A**) Map of non-CDC HPV56 (HPV56/pT713) plasmid. The L2 gene is split into two regions of the vector backbone. (**B**) Map of CDC HPV56 (HPV56/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S14. (A) Map of non-CDC HPV59 (HPV59/pUC9) plasmid. The E6 gene is split into two regions of the vector backbone. (B) Map of CDC HPV59 (HPV59/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S15. (A) Map of non-CDC HPV66 (HPV66/pBR322) plasmid. (B) Map of CDC HPV66 (HPV66/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S16. (A) Map of non-CDC HPV68 (HPV68/pBluescript SK-) plasmid. (B) Map of CDC HPV68 (HPV68/pGEMT Easy-01) whole genome plasmid without interruption of coding genes.



Figure S17. PCR with PGMY primers using CDC HPV whole genome plasmids as DNA template. Lane 1 and lane 11: DNA standard, lane 2: control PCR reaction without DNA template and lane 3: PCR reaction with empty vector backbone, and remaining lanes correspond to PCR reaction with CDC HPV plasmids resulting in ~450 bp PCR amplicon.

	~ DNA yield from	
CDC Plasmids	300 mL <i>E.coli</i> culture (μg)	
HPV6	665	
HPV11	770	
HPV16	320	
HPV18	145	
HPV31	380	
HPV33	330	
HPV35	250	
HPV39	435	
HPV45	660	
HPV51	480	
HPV52	390	
HPV56	315	
HPV58	630	
HPV59	770	
HPV66	510	
HPV68	278	

 Table S2 DNA yield from large scale purification of CDC HPV plasmids