Supplementary Figure S1

Late		PEN			Early
ΔΤ	-rich		EP	Site A	
1		<u>P1</u> P2_P3			
I≪— 8	<u>3</u> → 11 →			0 -1	
MuPyVCTGTTTT					GA <mark>GGC</mark> TTCCA <mark>GAGGC</mark> AACTTGTC-
LPyV CTAG TTTT					GCTTTTCCAAAA
MfasPyV1 - CCCTGACTT					GCTTTTCCAAA
VervetPyV1 - GAGGCTTTT					G CACTTACTTGCTTGCAGGAGGCG
HaPyV - TAGCATITT					GGGGCTTTTTGGCTTTAA <mark>GCCTC</mark> A
ChPyV - ATGCTTTTT HPvV9 CACAGGTTTTT					GGGACACTTGCACTTACTTGCTTC GCTTTTCCAAAACTCATTAAGGTA
ChaerePvV1 AAGC					TCTGCTACTTACTTGCAGGGTGTT
OtoPyV2 CAAGGTC					GTTCATACTTACTTGGACTAGTTC
PtrovPvV5 TAGGTTT					GCTTTTCCAAAACTCATTAAGGTA
PrufPyV1 AGAGGCTTTT					GCACTTACTTGCTTGCAGAAGGCG
MCV GTGACTTTTT					GGCTTAAGAGGCTTAATTAGCAAA
OtoPvV1 - GCTCTTTTT					AGGCTTTTTTTAAATTATAGCCGT
MinioPvV CCTTTTTTTT					AGGCTTTTTGGGGGGCTTTTTAGCA
HPyV12 CACCTCCTTT	TTTTTTTATTACTGG	GAGGC <mark>AA</mark> GAGGC <mark>ACC</mark>	CAGCCCCCGTCCTTCCTATA	AAAAAGGGAGAGGCTTTTGGA	GGCCATTTTTGGTACCAGGCACGT
CardiPyV GGGG TTTTTT	T T T T T T A A A CA GA GA	G A G G C <mark>G A </mark> G A G G C <mark>A C C</mark>	CG <mark>G C C T C C G A G G C T T T T G A A</mark>	A A <mark>T A A A</mark> GGA GA <mark>GA GG C C T T</mark> CA T	T T T G A G T A A T T A G T G C A C C T A A G G
GggPy1 CCTATTTTT	T T T <mark>A CA</mark> AAT TAGTGC	G A G G C <mark>T T</mark> G G G G C <mark>T C C</mark>	; T A <mark>G C C T C <mark>C G A G G C C T </mark>C <mark>T G G</mark> A</mark>	A A A A A <mark>T</mark> A <mark>G T G A G A G <mark>G C C T C</mark> T <mark>G</mark> A</mark>	G G C C T C T A A C A G C T T A A T T A G C A G
MasPyV GGATCA TTTT					A G G C T T T T C A G A G G C T T G C T C C T G
PtrosPyV2 A CCCTG TTTT					GCTTTTCCAAAACTCATTAAGGTA
MPtV TCA TTTT					CTTTTCAGAGGCTTTCCGAGGCTT
BPyVTGTAATTT					G G C G C C C C T A A A A G A G G A A A A T G
HpyV10 GCTAAAATTT					ATCTTTACCGCGTCTGCAAACTTT
STL_MA138CCTTTTT				CAGAGTTGACTCTGCAGCA	
BatPyV AGCATTTTTT					G CA CA A GGGGC TTTTCA GA GG CTT
EPyV AAACTTTTTT KIPyV TGCCCTTT					GCAAATAAGAAAA <mark>GCCCC</mark> GGGGGC GGC <mark>ATTTGAGAGGCCTAGAGAGGC</mark>
WUPVV CTTAGCTTTT					AGGCGCCTTGAGAGGCCTAGAGAGGC
PtrovPvV3CTAAGTTTTT					GGCTTGCAGGCAATTTTTCCTGTA
PtrovPvV4 - GAAGTCTTT					GGCTTTTGAGGCAATTGCTTTCAA
PterPyV - TTAACTTTT					CAAATATTTTGCAGCTACAGGCAG
OraPvV CAGTTTTT					C C T C T T G A G G C T T G G C A C A T C C T A
ApanPyV1 ATGCTTTT					G C C T C A G G A G G C C T G G C C A A C T G C
TSV TCTTGTTTTT					G C C T C T T G A G G C T T G G C A C T T C C T
EidoPyV1 TTT	TTTAAATTTTAAGTA	G A G G C <mark>A</mark> G A G G C <mark>T C T A</mark>	GGCCCCC <mark>GGAGGC</mark> TTTTG <mark>AA</mark>	A T A A A A A <mark>G A G A G G C</mark> T T C T <mark>G G G G</mark>	C C T G T G C A A A A C G G T C A G G C G G G A
CeryPy1 GGCCCTTTT	ΤΤΤΤ <mark>ΑΤ</mark> ΤΑΤΑΤΑΤΑΑ	G A G G C <mark>C</mark> G A G G C <mark>C G C C</mark>	CTA <mark>GCCTC</mark> CACCCT <u>T</u> TCTCA	C <mark>A A</mark> G T <mark>A</mark> G TA A G <u>GG TG T</u> G <mark>G A GG C</mark>	T <u>TTTTCT<mark>GAGGC</mark>CTAAAAAAGCCG</u>
SV40 CTGACTAATT	TTTTT <u>T</u> ATTTATGCA	G A G G C <mark>C</mark> G A G G C <mark>C</mark> G C C	TC <mark>GGCCTC</mark> TGAGCT <mark>A</mark> TTCCA	G <mark>A A</mark> G T <mark>A</mark> G T G A G <mark>G A G G C</mark> T T T T T T	G <mark>GAGGC</mark> CTAGGCTTTTGCAAAA
JCV CCTTCCCTTT					G <mark>GAGGC</mark> CAGGGAAATT
SV12 GCCTTTTT					TTTTTCT <mark>GAGGC</mark> CTAAAAAAATT-
BKV TGGCCTTTTT					T T T T T C T <mark>G A G G C</mark> C T A G C A A A A C T A
SLPyV1 TCAGAA					TGGCCCTTCAGGCTTTCAGCACCT
CalbPyV1 - CCCCA TTTT					GGGCCTGCGCAGCGGACCTACAAT
SqPyV TTTCCCATTT					GAGGCTGCAACTCAGCACCTGAAA
SsciPy1TTTCCCA TTT					GAGGCTGCAACTCAGCACCTGAAA
HpyV7 CACCTTT					GGTGTCAGCTGCACCTGCCACCAC
HPyV6 TCTCCT EelPvV CCTTTTT					TGGCCCTGCCCAGTTGCGTAAGCC TTTCAAATCAATC
PethAn CC	GTATGGAAGGAG	ACCICCUTCI		a lagiadia da lagia lagia lagia	

Figure S1. Nucleotide sequence alignment of 46 polyomavirus origins of replication.

The late and early sides of the origins are indicated. The AT-rich, the central pentamer sequences (PEN), and the EP regions are indicated. The adjacent site A region is also indicated. The sequences were manually aligned based on the P1 GAGGC pentamer. G(A/G)GGC sequences and their reverse complement are colored blue and green. Imperfect GAGGCs in P1 are indicated by highlighting the nucleotide in purple. Poly(T) sequences on the late side of the origin are colored magenta. Adenosine nucleotides on the opposite side of the PEN origin are colored red. The name of the polyomavirus, its abbreviation, and its Genbank accession number is given in Supplemental Table 2.

Supplemental Figure S2.



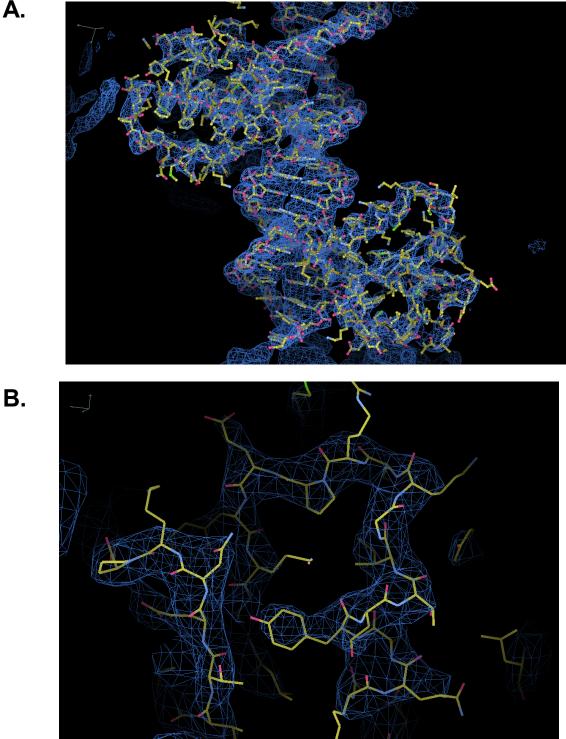


Figure S2. 2Fo-Fc Electron density from the Py DNA/OBD complex. In both figures the density was calculated using Coot and it is displayed at approximately 2 sigma in Coot. No density masking has been applied.

A. Electron density for much of the DNA as well as the origin binding domains bound to sites P1 and P2. The unoccupied P3 is also shown. The large solvent channels in the crystal are evident in this figure.

B. A blowup of some of the density in the obd.

Supplementary Table S1

	Py OBD + 26mer ORI Duplex DNA		
Data collection	$\lambda = 1.075 \text{ Å}$		
Space group	C 2 2 2 ₁		
Cell dimensions			
<i>a</i> , <i>b</i> , <i>c</i> (Å)	165.150 167.922 77.728		
α, β, γ (°)	90.0 90.0 90.0		
Resolution (Å)	50.00 - 3.79 (3.94 - 3.79)		
$R_{ m merge}$	17.0% (75.9%)		
Ι / σΙ	28.2 (1.4)		
Completeness (%)	99.83 (99.89)		
Redundancy	14.3 (14.6)		
Refinement			
Resolution (Å)	46.93 - 3.79		
No. reflections	11,080		
Completeness (%)	99.89% (99.53%)		
$R_{ m work}$ / $R_{ m free}$	0.2218/0.2444		
R.m.s. deviations			
Bond lengths (Å)	0.005		
Bond angles (°)	1.035		

X-ray Data Collection and Refinement Statistics.

Values in parentheses are for the highest resolution bin.

Supplemental Table 2

Polyomavirus name	Abbreviation	Host	Accession
African green monkey polyomavirus	LPyV	African green monkey	NC_004763
Ateles paniscus polyomavirus 1	ApanPyV1	Red-faced spider monkey	NC_019853
BK polyomavirus	BKPyV	Human	NC_001538
Bovine polyomavirus	BPyV	Bovine	NC_001442
California sea lion polyomavirus 1	SLPyV1	Sea Lion, California	NC_013796
Cardioderma polyomavirus	CardiPyV	Bat, Heart-nosed	NC_020067
Cebus albifrons polyomavirus 1	CalbPyV1	White-fronted capuchin	NC_019854
Cercopithecus erythrotis polyomavirus 1	CeryPyV1	Red-earded guenon	JX159985
Chaerephon polyomavirus 1	ChaeroPyV1	Bat, Free-tailed	NC_020065
Chimpanzee polyomavirus	ChPyV	Chimpanzee	NC_014743
Eidolon polyomavirus 1	EidoPyV1	Bat	NC_020068
Equine polyomavirus	EPyV	Equine	NC 017982
Gorilla gorilla gorilla polyomavirus 1	GggPyV1	Gorilla	HQ385752
Hamster polyomavirus	HaPyV	Hamster	NC 001663
Human polyomavirus 12	HPyV12	Human	NC_020890
Human polyomavirus 9	HPyV9	Human	NC_015150
Human Polyomavirus HPyV6	HPyV6	Human	NC_014406
Human Polyomavirus HPyV7	HPyV7	Human	NC 014407
Japanese eel endothelial cells-infection polyom		Japanese Eel	NC_015123
JC polyomavirus	JCPyV	Human	NC 001699
KI polyomavirus	KIPyV	Human	NC_009238
Macaca fascicularis polyomavirus 1	MfasPyV1	Crab-eating macaque	NC_019851
Mastomys polyomavirus	MasPyV	Mouse	AB 588640
Merkel cell polyomavirus	MCPyV (MCV)	Human	NC 010277
Miniopterus polyomavirus	MinioPyV	Bat	NC_020069
Murine pneumotropic virus	MPtV	Mouse	NC_001505
Murine polyomavirus	MuPyV	Mouse	NC 001515
MW polyomavirus	HPyV10	Human	NC_018102
Myotis polyomavirus VM-2008	BatPyV (MyPyV)	Bat	NC_011310
Orangutan polyomavirus	OraPyV		NC_013439
Otomops polyomavirus 1		Orangutan Bat	NC_010439
	OtoPy1	Bat	
Otomops polyomavirus 2	OtoPy2		NC_020066
Pan troglodytes schweinfurthii polyomavirus 2	PtrosPyV2	Eastern Chimpanzee	NC_019858
Pan troglodytes verus polyomavirus 3	PtrovPyV3	Western Chimpanzee	NC_019855
Pan troglodytes verus polyomavirus 4	PtrovPyV4	Western Chimpanzee	NC_019856
Pan troglodytes verus polyomavirus 5	PtrovPyV5	Western Chimpanzee	NC_019857
Piliocolobus rufomitratus polyomavirus 1	PrufPyV1	Eastern Red Colobus	NC_019850
Pteronotus polyomavirus	PterPyV	Bat	NC_020070
Saimiri sciureus polyomavirus 1	SsciPyV1	Squirrel Monkey	JX159989
Simian Agent 12	SA12	Vervet monkey, Baboon	NC_007611
Simian virus 40	SV40	Rhesus Monkey	NC_001669
Squirrel monkey polyomavirus	SqPyV	Squirrel Monkey	NC_009951
STL polyomavirus	STLPyV	Human	NC_020106
Trichodysplasia spinulosa-associated polyoma		Human	NC_014361
Vervet monkey polyomavirus 1	VervetPyV1	Vervet monkey	NC_019844
WU Polyomavirus	WUPyV	Human	NC_009539