



# National Institute of Standards & Technology

## Certificate of Analysis

### Standard Reference Material<sup>®</sup> 2366

#### Cytomegalovirus (CMV) for DNA Measurements

This Standard Reference Material (SRM) 2366 is intended primarily for use in the value assignment of the number of amplifiable genome copies of cytomegalovirus (CMV) per volume, such as copies per microliter. This material is to be used to provide traceability of calibration materials used in quantitative measurements of viral load in tissues or fluids such as plasma. The DNA consists of a bacterial artificial chromosome – known as CMV Towne  $\Delta_{147}$  BAC – that contains the genome of the Towne strain of CMV [1]. The three component DNA materials, labeled A, B, and C, are solubilized in TE buffer, pH 8.0, which consists of deionized water with 10 mmol/L 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) and 1 mmol/L ethylenediaminetetraacetic acid (EDTA) tetrasodium salt. A unit of SRM 2366 consists of three sterile 0.5 mL perfluoroalkoxy (PFA) fluoropolymer vials, each vial containing approximately 150  $\mu$ L of DNA solution of each individual component.

**Certified Values:** Table 1 lists the certified number of amplifiable CMV genome copies for each component. These certified values were determined by digital polymerase chain reaction (PCR) using the CP1 assay [2]. The values and uncertainties of the components A, B, and C were computed using a Gaussian random effect model [3]. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [4].

**Sequences:** Table 2 lists the regions of the CMV genome that were sequenced. The regions that were sequenced were those used as targets in published detection methods, commercial detection methods, and one gene (UL97) which when mutated can cause resistance to treatment with ganciclovir. Sanger sequencing was performed in both the forward and reverse directions, with overlapping reads, and verified by two analysts. Users of SRM 2366 can compare the oligonucleotide primers and probes specific for their CMV PCR assays with the sequence information provided following the tables.

**Expiration of Certification:** The certification of **SRM 2366** is valid, within the measurement uncertainties provided, until **09 July 2013**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

**Maintenance of SRM Certification:** NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical measurements leading to the certification was under the direction of M.J. Holden and J.M. Butler formerly of the NIST Biochemical Science Division.

R.J. Haynes and M.J. Holden prepared the materials and conducted measurements. M.C. Kline and M. Coble of the NIST Biomolecular Measurements Division, and D.L. Duewer of the NIST Chemical Sciences Division provided expertise and critical review, and assistance was provided by other members of the former Applied Genetics Group of the Biochemical Science Division.

Statistical analyses were performed by B. Toman of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Michael J. Tarlov, Chief  
Biomolecular Measurements Division

Gaithersburg, MD 20899  
Certificate Issue Date: 03 August 2015  
*Certificate Revision History on Last Page*

Robert L. Watters Jr., Director  
Office of Reference Materials

## NOTICE AND WARNING TO USERS

**Storage:** All vials of SRM 2366 should be stored in the dark between 2 °C to 8 °C. **DO NOT FREEZE.**

**Handling:** SRM 2366 IS A VIRAL AND BACTERIAL SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE THE SRM 2366 COMPONENTS AS BIOSAFETY LEVEL 1 MATERIALS CAPABLE OF TRANSMITTING INFECTIOUS DISEASE [5]. SRM 2366 components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

## INSTRUCTIONS FOR USE

Component vials should be mixed briefly and centrifuged (without opening the vial cap) prior to removing sample aliquots for analysis. For the certified values to be valid, materials should be withdrawn immediately after opening the vials and processed without delay. Dilutions of these materials may be made as appropriate, but they must be used immediately. Certified values do not apply to any material remaining in recapped vials. **DO NOT EXPOSE ANY DNA SOLUTION TO DIRECT SUNLIGHT.**

## PREPARATION AND ANALYSIS

**Sample Preparation:** The CMV Towne<sub>Δ147</sub> BAC was obtained from the New Jersey Medical School (Dr. Hua Zhu, Newark, NJ) and was propagated at NIST in *Escherichia coli* cells. BAC DNA was isolated, purified, and resuspended in TE buffer. After concentration adjustment, the materials were allowed to equilibrate at 4 °C in PFA containers until vialing. Just prior to vialing, each sequence was brought to room temperature inside a laminar flow hood and gently mixed. In separate sessions, each solution was transferred to sterile PFA vials with a manual pipette, which were then capped and labeled. After each production run, all vials were placed into storage at 4 °C.

**Material Quantification:** Quantification of SRM 2366 was accomplished using digital PCR. DNA in PCR master mix was prepared so that once the DNA solution was distributed to an array, many of the chambers had 0 or 1 copy of the amplification target. A correction to account for the chambers with more than one copy of the DNA PCR target was done using Poisson statistics. Each component of SRM 2366 was quantified separately using three randomly selected vials. Assays were conducted on each vial using 3 separate arrays (or 25 000 separate reactions). Six PCR assays targeting different regions of the CMV genome were tested and the results were not found to be significantly different. A single assay [2] targeting the CMV DNA polymerase (UL54) was used for quantification of all three components.

**Sequencing:** Sanger sequencing was performed and the products were separated using capillary electrophoresis. Sequence reads were analyzed by two independent analysts.

**Short-Term Stability Assessment:** Vials from each component were subjected to 22 °C, 37 °C, or 65 °C for a period of one to four weeks with the control at 4 °C. Isosynchronous quantitative PCR (qPCR) assays were performed at the end of 4 weeks and cycle threshold (Ct) values were analyzed. The results show that the material is stable after four weeks at 22 °C and 37 °C, but not at 65 °C.

**Homogeneity Assessment:** Vials were selected from each lot (100 consecutively filled vials) of each component to test for homogeneity that could be caused by bulk heterogeneity or changes during distribution of the DNA solution to vials. All tubes were assayed in triplicate using qPCR. No systematic trend between lots was observed. The random effects model [3] used to determine the values and uncertainties of the SRM components accounts for the variability between lots.

**Commutability of SRM 2366:** Vials of Component B of SRM 2366 were provided to Quality Control for Molecular Diagnostics (QCMD) to be included in their 2010 CMV external quality assessment (EQA) program. QCMD test materials were sent out to the CMV EQA participants. The other materials provided to participants consisted of ten lyophilized samples with different concentration of CMV virus (AD169 strain) in plasma or viral transfer medium. The NIST component B material was supplied as an unknown and came with instructions to directly add the CMV DNA to the participant's assay without extraction. A total of 179 data sets were submitted by participants for SRM 2366. Laboratory-developed assays were used to generate 80 datasets and commercial assays were used to generate 99 data sets. The results for SRM 2366 provided evidence that SRM 2366 is commutable with most assays that are in current use.

Table 1. Certified Values of the Number of Amplifiable CMV Genome Copies

Component	Value (copies per microliter)	Standard Uncertainty (copies per microliter)	Relative Uncertainty (%)	Expanded Uncertainty 95 % Confidence Interval (copies per microliter)	
A	420	56	13.3	301	to 523
B	1702	130	7.6	1446	to 1959
C	19641	365	1.8	18924	to 20359

**Sequences:** The nucleotide numbering is based on the Towne sequence as deposited in GenBank (AY315197.2 Human Herpes Virus 5 Towne strain). There is complete agreement between the NIST sequence and the GenBank accession, AY315197.2, with one exception at the position identified below. Table 2 lists the nucleotide ranges that were sequenced.

Table 2. Exact Nucleotide Ranges That Were Sequenced for CMV BAC.

Reference Sequences	Nucleotide Range	# Bases Sequenced
UL34	43202 to 44971	1770
UL54 <sup>(a)</sup>	77695 to 79992	2298
UL55 to 56	80848 to 82731	1884
UL80	114401 to 116793	2393
UL83	118890 to 119937	1048
UL97	140784 to 142090	1307
UL122 to 126	170525 to 173182	2658
UL132	176380 to 177192	813
US17	198929 to 199312	384

<sup>(a)</sup> The sequence exception at position 78651 (UL54) is described as a Y (C/T) at NIST and a C in GenBank.

**UL34 nucleotides 43202 to 44971:**

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CAAAC GAAGC GTGTC TTTCA ATTTT CCCAG CGGTA CGTGG AAAGG CGGTC AGAAA ACCGC GTCCA
ACGAC ACATC CACAA AAATC CCCCA TCGAC TCTCA CAATC GCATC ATAAC CTCAG CGGGG TATGA
GCTTT CCTGT TACTT TATT CAGAAA GCACC AGAAC CCGTC GCCAT TTCCC CTCAT ATACG GTACA
CGTCC CCCTG ATCTG TCATC ACGGT ACACA GATTT CGCCC GACTG CGGAC ACCGA CGGCC AATCG
CGTGG CGTAG GAGTG GCGCC CCGGC TTCAT TATAA CGCCA CGTCG GAGCC CCTGC GCGCC ACAAC
GCCGT CCGGC GCAAC TTCTG TCTCG GCACG GTACG ATAAA AACGA CGTCC CCCGT CGACG TTGTT
TTCTC CGAGC GGTGA TCGTT CCCGT CCCTC TCCTC CCTCC GCGGC CCCCC CGGCG CGGCC CCGCT
CGCAC GGACC TATAC TATTA CCGCC CCACC GCCGT CGTCG TCATG AACTT CATCA TCACC ACCCG
AGACT TCTCC AACGA CGATT CAGTC CTGCG AGCCG CCGAG ATGCG TGACA ACGTG GCAGG CTCGA
TTTCC AAAGC GTACA AGGGT ACGGT ACGCG CCGAA GGCAA GAAGA AGCTG CTGCT GAAGC ACTTG
CCCGT GCCGC CCGGC GGCTG CTCGC GCCGC AACAG CAACC TCTTC GTTTT CTGCA CCGAG CGCGA
CTACC GCAAG TTCCA CCAGG GCATC GCACA GCTCA AGCGC GCGCC GGCCG AACTG GACCC CCACG
AGATC CAGCA AGTCA CGGCC AGTAT CCGCT GCCGC CTGCA GCCCA GTCTC CGCGA GCCGC CCACG
CCGGC CGACG AGCTG CAGAC GGCTG TGTCG CGCGT GTGCG CGCTC TTCAA CCAGT TGGTT TTCAC
GGCCC AGCTG CGCCA CTA CTACT GCGAG CACCA GGACA AGGTG GTGAG CTACG CGCGC GACGA GCTGA
CTAAA CGCTG CGGCG AAAAA TCGGC GCTGG GCGTG GAGGT GCATC AACTG GTAGC CCTGC TGCCA
CACGA GCGCC ACCGC GAACT GTGCC ACGTC CTCAT CGGCT TGTTG CACCA GACGC CGCAC ATGTG
GGCGC GCTCC ATCCG TCTCA TCGGA CACCT GCGCC ACTAC CTGCA GAACA GCTTC CTACA CCTGT
TGATG AACTC AGGTT TGGAT ATCGC GCAAG TCTTC GACGG CTGTT ACCAC AGCGA GGCCT ACCGC
ATGCT CTTCC AGATC GGTCA TACGG ACTCG GTGTC GGCGG CCCTG GAACT TTCAC ACAGC GCGGC
GGCCG GGCCG CCCGA GGCCG ATGAG AACAA CGACG AAGGA GAGGA GGACG ACGAC GAGCT CCGTC
ACAGC GACCC GGCGC CGCTT CACGA GTCCA AGAAG CCCC GCAAC CCCGT CGTCC CCGCA CACGC
GTGCC GCCTC ACGAG CAAAA GCCCG AAGAA AACGA GGAGG AAGAA GAGGA GCTGT TTCCC TCCTG
CAAGG CAACC GCAGC ATTCC TGCGG GCAGA ACCCT CCGTC TCCAA CGACG ACGGC AACGG CGGCG
AACGC TGCGA CAGC TAGCG ACCCG CCTGC GGCAT TGCGC CGACG AAGAA GACGG ACCTC TAGCC
AGCCA GACCG CTGTG CCGGT CGCCG CGACC CCCTC ACCTT CAGTC ACCCC AGCCC TTACC CCCGT
CACGT CCCCC ATAAC CCCGT TGTGT ATTTA ACGTC ACTGG AGAAC AATAA AGCGT TGATT TCTCA
AGTTC CGCTC TGGTT
    
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**UL54 nucleotides 77695 to 79992:** The sequence exception at position 78651 is enclosed in a box.

ACTTT GAGCG CCATC TGTTC CTTGT CGAGC AACAT ACGAC GCACA GGGTC TTGAC ACTCG CGCAT  
GCATT CGCGC ACGGC ACGCC GCTGC GAAAC CCACT TGTTG AGCAG TTCCG AAAGC ACCGA GACGC  
GCACC GAAGC GCGCA CAAAG CGGTG GGTCA CGCCG TTCTC TAGCG TGACG CTGTA TACGT CGGCA  
GGGTC CACGG GGTAC TCGCC ACCCG GCACC AACAG GGTGG AGTAG CAGAG GTTGT GGGCC ATGAT  
GATGG AAGGG TAGAG GCTGG CAAAA TCGAA CACGG CCACG GGGTC GTTGT AGTAA CCCAC CTCGG  
GCTCA AACAC CGTGG CACCT TGGTA CGAAA CCGCC GCGGT ACCGC CGGCG CCGTG ACTGT CGTTG  
GAAAC GCCGA CGCCG CCACT ACTGC CGGAG CCGAC GCTGA AAACG CCGAC GCTGC TACTA CTGTT  
ACTAC CAGAG CCGGG TGAAA CGCCG TCCTG ACTCG ACGGC GCAGA TTGCA AGGGC GGCGA CATCT  
GAAAC ATAGC CGCCA CAGAA CCCGC GTCGC CGGGC ACGGC GCGCG TAGAG ATGAT AGCGA CGTTA  
GGTGA CACGG CAACG CTATT CGTTT CGGGC ACCGT CCGTAC CTTTG CTGTA GTGGT TGGGC AAGAT  
AAAAT CGCGG CAGGC CCACT CGTCT AGCAG CGAGG TGTAG ATACG GATCT GCTGT CCGTC AAAGA  
TGACA CGCCG CAACG GAATT TTAGC CAGCC GCGCG ATGGC CCCGG CCTCG TAGTG AAAAT TAATG  
GTGTT GAACA GATCG CGCAC CAATA CGGCG TCCTG CAGAC AGTAA CGGCC TACCT GGGCG CGGCC  
CTCGG CATTA GCCAC GAAAC AACGC GGGAT GTCCT TGTAG GACAG GTCAT CCTTG CGTTG CCGCA  
GGTAA AGCTC GGCCA TAGTG TTGAG CTTAT AGTTG GGCGA GTTAG TTTG GCCAT GCATA CGGGG  
TACAT GTCGA TAACC ACCGA ACCCG CAATA TACAC CTTGG TGGCG GCCGT GCTGG CCGGA TTATT  
GTGAG AAGCC GAGGG AAAGG CGGCG GCGTA CTGCC GCTTA AAACC CACGG CGGGG CTGTG TAAAA  
AAAAA CGGCC GCCCT GCGCC GTGGG CAACT TGCAG AAGCG CTGCG AGTCC ACCTT ATACA GGTAC  
TCGAG GCGCG TGAGG ATGTA CTTCA AGTCA AAAGA GTTGA TGTTG TAACC GGTCA CAAAG GCCGG  
CGCGT ACCGT TGAAA GAAAA GCATA AAGCC CAGCA GCAGC TCGTA TTCGG AAGGG AACTC GTAGA  
CGTCT ACGTC TGGGC CCACC TGCCC GCAGG TGCCG ATCGT AAAGA GATGA AGACC CGAGT GCCCA  
AAGAT CACGC CCTCC GAGGT GCAGC CCCGA CCATC GTTCC CGTTT GGGAT TCCCT GATCC ACGGC  
GGTGT TTCCT CCCGT CTCGT AGCAC ACGCA CGAGA TCTGA ATGAC AATGT CATCA GACTT CTCGG  
CGCAG GGAAA ACCAC CCTCG CCGCT CATGC ACTCG ATATC GAAGG ACAGG CACCG ATAAC GCGGC  
CACGA GCTGT CGTCG GGCAC GGCCA CCAGG TCGGA GACAT CGCAG TCGAC CTCGA TATCA CAAGT  
CGACG CGCGA CCCTG CTGCC GCCAG TCGTA ACGAT TCACG GAACA CCAGC CGAAC GTGGT GATCC  
GCCGA TCGAT GACCA AACGC GTCAG CGGAT CCACA CGGAC CTCGT ACACG GGAAA ACCCT GCTCC  
AGCAG ATACT CGCCG ATCTT TCTAG CCATG GTCCA GTTGC TGATA GACAC ACACT GCAAA TCGGG  
CACGG GTCGC GTCCC GTACC CGTAG ATGGA GGTCT TGGTG GCCGG CGTGA CAGAC ACGGC GTATG  
GCGTC CGCGG TTCGG GCACT AGTTC GCCCA CGCTG GCAAT GACCT CACGC AGCCT ATCGG TGTCC  
CTGTA CTCAC AGTAA AAGTA GCTGC GCTGC CCGAA AACGT TGACG CAGAT ACTGT AGCCG TGTTT  
TGTGG CCCC GAGAA ACGCA ACACG TTCCC CGAAG GCACC AGATG CTGAC GATAG CGCGG CGACA  
CGTTT TCGGG CGAGT CGAAG AAGAG CACGG CGTCC GTTTG ATCGT AGGTG TGAAA ACGAA TAGGT  
CCCAC CACGC GACCC ACCAG GGTCT CGCGC CAAGG ACACG GCCAA ACCAT GTCAT GACTC AACAA  
ATGTT TAATC TCTCG ATAGA ACATG AGAGG CAACC GTCCC GTCTT ATGCT TGATC AACCC CGTCT  
GACCC TCGAA CATGA CGCCT CGC

**UL55 to UL56 nucleotides 80848 to 82731:**

GAGGT CGTCC AGACC CTTGA GGTAG GGCGG CAGCG GGTCG ACTAC CTTGT CCTCC ACGTA CTTTA  
CCCGC TGCTT ATACG AATTG AACTC GCGCA TGATC TCCTC GAGAT CAAAA ACGTT GCTGG AACGC  
AATTC TTTCT GCGAG TAAAG TTCCA GTACC CTGAA GTCCG TGTTT TCCAG CGGGT CGATG TCTAG  
GGCGA TCATG CTGTC GACGG TGGAG ATGCT GCTGA GGTC AATCAT GCGTT TGAAG AGGTA GTCCA  
CGTAC TCGTA GGCCG AGTTG CCGGC GATGA AGATC TTGAG GCTGG GAAGC TGACA TTCCT CAGTG  
CGGTG GTTGC CCAAC AGGAT TTCGT TATCC TCGCC CAGTT GACCG TACTG CACGT ACGAG CTGTT  
GGCGA AATTA AAGAT GACCA CTGGT CGTGA GTAGC AGCCT CCTGG CGATT CCTTC ACATT CATAT  
CACGC AGCAC CTTGA CGCTG GTTTG GTTAA TGGTC ACGCA GCTGG CCAGA CCCAG GACAT CACCC  
ATGAA ACGCG CGGCA ATCCG TTTGT TGTAG ATGGC CGAGA GAATA GCTGA CGGGT TGATC TTGCT  
AAGTT CCTTG AAGAC CTCTA GGGTG CGCCG TTGAT CCACA CACCA GGCTT CTGCG ATTTG CGCCA  
GCGCC CGGTT GATGT AACCG CGCAA CGTGT CATAG GTGAA CTGCA GCTGG GCGTA GACCA GATTG  
TGCAC CGACT CCATG TTGGA TAAAT GAGTT GCATT GTTGC CATCT GACTT TCTTT TGGTT CTATT  
ATGAG TAAGA TTCAG ACTGG AGCGG TTGGC CAAAC GTTCG AGTTC CACCA GAGAT TTTTG CTTGA  
TACCT TGCCA GAACA CCACC AAACC ACCAG TGGTT TCAAA GACGG ACACG TTTCC ATATT TTTCA  
TATGT TTGAT TGTAT GAAGT ATTGA AAATC TGCTG TAACT TATTT ATGGC CTCAT CACGT ACACA  
GTCCA GCGCA GAGTC GGACA TGTTC ACCTC TTGCT TCTTA GATAA GAAAG TGGCG GTCAT TTTGG  
CAGAA GAAAA GTGAT ACGAG TCCTC GGCTT CGGAA CGAAT GGTGC GTTCC GAGGC TTCCC AGAAA  
GTGAG TTGAC AAGTA ACATT CTTCT CGTCC TGTAT ATCCC AGGAG ATCAC TGAGT CCGCA CGTTC  
AAGAA AAGCC ACCAA CCTGT GGGTC TCTAA CGCAG AATTC GGTCT TCCAA AGTCG GAGAC GATAG  
TGTAG TTCGG AAAAA TGAAA AACTT GTCGG CGTTT TCTCC AAAAT AGCTG GCATT GCGAT TAGTT  
CCGTT GTAGA AAGGA GAAAT GTCAA CCACA TCACC CGTGG AAGTT GCGAA AAAAT GATAG GGATA  
CTTGG AGCGC GCAGT AGTGA TGGTC ACCAT ACAAT TCAGA TTACA GGTCT CACGA TAGAG CCAGG  
TGCTG CCGCG GCTGT GCCAT TGATC CTTGA CCGTC ACGTA ACGGG TACTG TGGGT GTTGG AATAA

TCGTC GGGCA TTAAT TGCAT GGTTT TGTTT TCATA GCTGT CCCTA TGATA AGCCA CGAAA ACCGT  
GCCTG CTATA ACGCG GCTGT AGGAA CTGTA GCACT GACTG TGACT GTTGA TATGA TGAAT CTCCC  
ACATA GGAGG CGCCA CGTAT TCCGT GTTGC TGCCC AGCAG ATAAG TGGTG TGGAT GTAAG CGTAG  
CTACG ACGAA ACGTC AAAAC CTCTT GGTAG ACTCG TACCT TAAAG GTGTG CGCGA CGATG TTGCG  
TTTGT AGACC ACCAT GATGC CCTCG TCCAG GTCTT CATTG ATGGG CTTCA TCGAG GTGCA GACGA  
TATTA CGTTC AAAGC GAATA AGATC CGTAC CCTGT GCCAT AGAAC ACACG CGATA GGGGT ACTT

**UL80 nucleotides 114401 to 116793:**

CGCGC AGTAG CAGCG TTTCG AGACC GCGGT GAAAG AGGAG GACGC AGATG AGGCG TACGA TTTTG  
AGTTC TTCCA AACGC AGCGA GCTCA GCGGC TGTCG GCGCG ACATC TTCTC GCTAA TCTGT AATAT  
TAGAT GATTG CCGCA AGTAA AGGAG AATTT GCCTG TGC GG GCGGAC ACCCG CGGCG GGGTT CTCTT  
CGTCG GGGC CATCA TCGTT CGCTC GGTGA GCGGG TAGCG ACGGT GACGA CAATG ACGAT GACGC  
AGCAG CAGCC GCAGG CTGTG ACGCC GGTCT ACGTG GCGCG CTTTC TCGCC CGTTA CGACC AGTCT  
CCGGA CGAGG CCGAA TTGCT GTTGC GCGCG GACGT AGTGG AGCAC TGGTT GCACG CGCAG GGCCA  
GGGAC AGCCT TCGTT GTCGG TCGCG CTCCC GCTCA ACATC AACCA CGACG ACACG GCCGT TG TAG  
GACAC GTTGC GCGCA TGCAG AGCGT TCGCG ACGGT CTTTT TTGTC TAGGT TGCGT CACTT CGCCC  
AGGTT TCTGG AGATT GTGCG CCGCG CTTCG GAAAA GTCCG AGCTG GTTTC GCGCG GGCCC GTCAG  
TCCGC TGCAG CCGGA CAAGG TGGTG GAGTT TCTCA GCGGC AGCTA CGCCG GCCTC TCGCT CTCCA  
GCCGG CGCTG CGACG ACGTG GAGGC CGCGA CGTCG CTTTC GGGCT CGGAA ACCAC GCCGT TCAAA  
CACGT GGCTT TGTGC ACGGT GGGTC GCGGT GCGCG TACGT TGGCT GTGTA CGGAC GCGAT CCCGA  
GTGGG TTACC CAGCG GTTTC CAGAC CTCAC GCGCG CCGAC CGCGA CGGGC TACGT GCACA GTGGC  
AGCGC TGCGG CAGCA CTGCT GTCGA CGCGT CCGGC GATCC CTTTC GCTCA GACAG CTACG GCCTG  
TTGGG CAACA GCGTG GACGC GCTCT ACATC CGTGA GCGAC TGCCC AAGCT GCGCT ACGAC AAGCA  
ACTAG TCGGC GTGAC GGAGC GCGAG TCGTA CGTCA AGGCG AGCGT TTCGC CTGAG GCGGC GTGCG  
ATATT AAAGC GGCGT CCGCC GAGCG TTCGG GCGAC AGCCG CAGTC AGGCC GCCAC GCCGG CCGCT  
GGGGC GCGTG TTCCC TCTTC ATCCC CGTCG CCTCC AGTCG AACCG CCATC TCCTG TCCAG CCGCC  
TGCGC TTCCA GCGTC GCCGT CCGTT CTCCC GCGCG AATCA TCGCC GTCGC TTTCT CTTCT GGAGC  
CGGCA GAGG GCGGT CCATG TCGCA CCCTC TGAGT GCTGC GGTTA CCGCC GCTAC GGCTC CTCCA  
GGTGC TACCG TGGCA GGTGC GTCGC CCGCT GTGCC GTCTT TAGCG TGGCC TCACG ACGGA GTTTA  
TTTAC CCAAA GACGC TTTTT TCTCG TFACT TGGGG CCAGT CGCTC GGCAG GCACC GTCAT GTATC  
CCGGC GCCGT AGCGG CCCCCT CCTTC TGCTT CGCCA GCACC GCTGC CTTTG CCGTC TTATC CCGCG  
TCCTA CCGCG CCCCC GTCGT GGGTT ACGAC CAGTT GGCGG CACGT CACTT TGCGG ACTAC GTGGA  
TCCCC ATTAT CCGGG GTGGG GTCGG CGTTA CGAGC CCGCG CCGTC TTTGC ATCCG TCTTA TCCCG  
TGCCG CCGCC ACCAT CACCG GCCTA TTACC GTCGG CCGCA CTCTC CGGGC GGTAT GGATG AACCA  
CCGTC CGGAT GGGAG CGTTA CGACG GTAGT CACCG TGGTC AGTCG CAGAA GCAGC ACCGT CACGG  
GGGCA GCGGC GGACA CAACA AACGC CGTAA GGAAG CCGCG GCGGC GTCGT CGTCC TCGGA CGAAG  
ACTTG AGTTT CCCC GCGAG GCCGA GCACG GCCGG GCGCG AAAGC GTCTA AAAAG TCACG TCAAT  
AGCGA CGGTG GAAGT GGCGG GCACG CGGGT TCCAA TCAGC AGCAG CAACA ACGTT ACGAT GAACT  
GCGGG ATGCC ATTCA CGAGC TGAAA CCGCA TCTGT TTGCC GCGCG GCAGA GTTCT ACGTT ACTTT  
CGGCG GCTCT CCCC CTGCG GCCTC TTCTT CCCC ACTAC TACTA CCGTG TGTA TCCCA CCGGC  
GATCT GACGA GCGGC GGAGG AGAAA CACCG ACGGC ACTTC TATCA GGAGG TGCCA AGGTA GCTGA  
GCGCG CTCAG GCCGG TGTGG TGAAC GCCAG TTGCC GCCTC GCTAC CGCGT CCGGT TCTGA GGCGG  
CAACG GCAGG GCCTT CGACG GCGGG TTCTT CTTC TGCCC GGCTA GTGTC GTGTT AGCCG CCGCT  
GCTGC CCAAG CCGCC GCAGC TTCCC AGAGC CCGCC CAAAG ACATG GTGGA TCTGA ATCGG CCGAT  
TTTTG TGGCT GCGCT CAATA AGCTC GAGTA AGAGA GACGC TATAT TTAGG GCT

**UL83 nucleotides 118890 to 119937:**

CTGCC ATACG CCTTC CAATT CCGCG AAGAT GCGGT AGATG TCGTT GCGGT CCCAG AAGAA CTCCT  
GGTAC TTCAG ATTCT GACCC TGAAC CGTAG CCACC ATGGG CACCA GGTG CCGGC CAGGA TGCCG  
GCCTG CCAGG GCGGC CAGGT GAACA CCGCC GGATT GTGGA TTTCG TTGTC GGAAT CCTCG TCGGT  
GTCCT CTTCG GGCGC GACGG TGGAC TCGGC CTAA GGCGG CCGCG TGTCA TAACG CCCGC CGTGC  
ACGCC GTCGC CGAGG ATGCT GATTT GCGTT TGCGG CCGCG GGAAG TGGAG GCGCC CGCCA TGGCG  
CCGCC GCCGG TAACG CCGGG CGTCT TGCGC TCGGT GGTTA CGAGT TCCTC GTCGG AGTCC GATCC  
GCTGG TCCAG ACGTC GTCGT CGCCC TGGGC GGCAC CCTCG TCGTG CCGGT CCCAG GTGTG TCGGT  
ACTCA AGCTT GCCCT GGATG CGATA CTGGC TGGTG AAGGT GGGGT GTTCG CTGTA CTGAG GCCCG  
CGCTG CAGCA GCAAG TCGAT ATCGA AAAAG AAGAG CGCAG CCACG GGATC GTACT GACGC AGTTC  
CACGG TCTCG CGTAT CGCTT GCACC TCCAG GAAGA TCTGC TGCCC GTTCA TCAAT AGGTT ACCTG  
AGATG CTCAG GCCCG GGATG CTCTT GGGAC ACAGC AGCCC AAAAT GCTCG TGTGA GGTA AAGCC  
ACATC CAGCA TGATG TGCGA GATCT TGCCC GTTTT GATTA TCATA TTTTT GGGAC ACAAC ACCGT  
AAAGC CGTTG CGCTC GTGGG GGCGC ATGAA GGGTT GCGGG TTGCG GGTCA TCGTC AGGTC CTCTT  
CCACG TCAGA GCCCA GCGTG ACGTG CATAA AGAGC TTGCC GGAGG GCACG TCCTC GCAGA AGGAC  
TCCAG GTACA CCTTG ACGTA CTGGT CACCT ATCAC CTGCA TCTTG GTTGC GCGCG TGTTC TCCAT

GGAGC AAACC AGCTC GTGCG CGCAC ACCAC GTGCC GCAGT GCCAC GTCCT TGGTG GGAAA CACGA  
ACGCT GAC

**UL97 nucleotides 140784 to 142090:**

TGCGA CCCGC GTATG TTCTT GCGCC TTACG CATCC CGAGC TCTGC GAGCT CTCTA TCTCC TACCT  
GCTGG TCTAC GTGCC CAAAAG AGGAC GATTT TTGCC ACAAG ATCTG TTATG CCGTG GACAT GAGCG  
ACGAG AGCTA CCGCC TGGGC CAGGG CTCCT TCGGC GAGGT CTGGC CGCTC GATCG CTATC GCGTG  
GTCAA GGTGG CGCGT AAGCA CAGCG AGACG GTGCT CACGG TCTGG ATGTC GGGCC TGATC CGCAC  
GCGCG CCGCT GGCGA GCAAC AGCAG CCGCC GTCGC TGGTG GGTAC GGGCG TGCAC CGCGG TCTGC  
TCACG GCCAC GGGCT GCTGT CTGCT GCACA ACGTC ACGGT ACATC GACGT TTCCA CACAG ACATG  
TTTCA TCACG ACCAG TGGAA GCTGG CGTGC ATCGA CAGT ACCGA CGTGC CTTTT GACAG TTGGC  
CGACG CTATC AAATT TCTCA ATCAC CAGTG TCGTG TATGC CACTT TGACA TTACA CCCAT GAACG  
TGCTC ATCGA CGTGA ACCCG CACAA CCCCC GCGAG ATCGT GCGCG CCGCG CTGTG CGATT ACAGC  
CTCAG CGAGC CCTAT CCGGA TTACA ACGAG CGCTG TGTGG CCGTC TTTCA GGAGA CGGGC ACGGC  
GCGCC GCATC CCAA CTGCT CGCAC CGTCT GCGCG AATGT TACCA CCCTG CTTTC CGACC CATGC  
CGCTG CAGAA GCTGC TCATC TGCGA CCCGC ACGCG CGTTT CCCC TAGCC GGTCT ACGGC GTTAT  
TGCAT GTCGG AGCTG TCGGC GCTGG GCAAC GTGCT GGGCT TTTGC CTCAT GCGGC TGTGG GACCG  
GCGCG GTCTG GACGA GGTGC GCATG GGCAC GGAGG CGTTG CTCTT TAAGC ACGCC GGCGC GGCCT  
GCCGC GCGTT GGAGA ACGGC AAGCT CACGC ACTGC TCCGA CGCCT GTCTG CTCAT TCTGG CGGCG  
CAAAAT GAGCT ACGGC GCCTG TCTCC TGGGC GAGCA TGGCG CCGCG CTGGT GTCGC ACACG CTGCG  
CTTTG TGGAG GCCAA GATGT CCTCG TGTCG CGTAC GCGCC TTTTC CCGCT TCTAC CACGA ATGCT  
CGCAG ACCAT GCTGC ACGAA TACGT CAGAA AGAAC GTGGA GCGTC TGTGG GCCAC GAGCG ACGGG  
CTGTA TTTAT ATAAC GCCTT TCGGC GCACC ACCAG CATAA TCTGC GAGGA GGACC TTGAC GGTGA  
CTGCC GCCAA CTGTT CCCCC AGTAA CCGGG ACGCG GAACG TGACG GTTGC TGAGG GGAAA GCGCA  
CAGAG AA

**UL122 to UL126 nucleotides 170525 to 173182:**

CAGCA CCATC CTCCT CTTCC TCTGG GGCAA CTTCC TCTAT CTCAG ACACT GGCTC AGACT TGACA  
GACAC AGTGT CCTCC CGCTC CTCCT GAGCA CCCTC CTCCT TCATC ACTCT GCTCA CTTTC  
TTCCT GATCA CTGTT CTCAG CCACA ATTAC TGAGG ACAGA GGGAT AGTCG CCGGT ACAGG GGACT  
CTGGG GGTGA CACCA GAGAA TCAGA GGAGC TGGCA CCAGC GGTGG CAAA GTGTA GGCTA CAATA  
GCCTC TTCCT CATCT GACTC CTCGG CGATG GCCCG TAGGT CATCC ACACT AGGAG AGCAG ACTCT  
CAGAG GATCG GCCCC CAGAA TGTA TGGGC AAAGA CCTTC ATGCA GATCT CCTCA ATGCG GCGCT  
TCATT ACACT GATAA CCTCA GGCTT GGTTA TCAGA GGCCG CTTGG CCAGC ATCAC ACTAG TCTCC  
TCTAA GACAT AGCAG CACAG CACCC GACAG AACTC ACTTA AGAGA GAGAT GCCCC CGTAC ATGGT  
CATCA TACAA GCGTC ACTAG TGACC TTGTA CTCAT TACAC ATTGT TTCCA CACAT GTAGT GAGGA  
TATCC ATAAA TATGT GATCA ATGTG CGTGA GCACC TTGTC TCTCT CCTCA TCCAA AATCT TAAAT  
ATTTT CTGGG CATAA GCCAT AATCT CATCA GGGGA GCAC T GAGGC AAGTT CTGCA ATGCC GCCAT  
GGCCT GACTG CAGCC ATTGG TGGTC TTAGG GAAGG CTGAG TTCTT GGTA AGAAC TCTAT ATTCC  
TGTAG CACAT ATACA TCATC TTCT CTTAA GTTCA TCCTT TTTAG CACGG GCCTT AGCCT GCAGT  
GCACC CCCC ACTTG TTAGC GGCGC CTTG CTCAC ATCAT GCAGC TCCTT AATAC AAGCC ATCCA  
CATCT CCGC TTATC CTCAG GTACA ATGTA GTTCT CATA ATGCT CTGCA TAGTT AGCCC AATAC  
ACTTC ATCTC CTCGA AAGGC TCATG AACCT TATCT AAGAT ATCTA AGGCA TTCTG CAAAC ATCCT  
CCCAT CATAT TAAAG GCGCC AGTGA ATTT TCTTC CGTCT GGGTA TATTT TTTC GCATG TGCTC  
CTTGA TTCTA TGCCG CACCA TGTCC ACTCG AACCT TAATC TGTTT GACTG TAGAG GAGGA TAACA  
ACACA TATAA GTATC CGTCC TCCTG ACTCA TTTAT CGCTA TCCTG ATGCC CCGT CACAT GCAAG  
AGTTA ATCTT TACTT TATCT GACAT ACACA AGTAA ATCCA CGTCC CATGC AGGTT AGTAT ACATC  
ACATA CATGT CAACA GACTT ACCGA GTTCT GCCAG GACAT CTTTC TCGGG GTTCT CGTTG CAATC  
CTCGG TCACT CGTTC AAAAG TTTG AGGGA TTCTT CCGCC AACTC TGGAA ACAGC GGGTC TCCCA  
GACTC AGCTG ACTGT TAACC TCCTT CCTCA ACATA GTCTG CAGGA ACGTC GTGGC CTTGG TCACG  
GGTGT CTCGG GCCTA AACAC ATGAG AAATA GAGTC ATAAG CACAT GGGTC ACATA CAGGA GATAT  
GTATA TAACA TTAAT ACAAT TTTAT AAAAA AAAGG GGGGG CACAA ACCCC GACAC GTACC GTGGC  
ACCTT GGAGG AAGGG CCCTC GTCAG GATTA TCAGG GTCCA TCTTT CTCTT GGCAG AGGAC TCCAT  
CGTGT CAAGG ACGGT GACTG CAGAA AAGAC CCATG GAAAG GAACA GTCTG TTAGT CTGTC AGCTA  
TTATG TCTGG TGGCG CGCGC GGCAG CAACG AGTAC TGCTC AGACT ACACT GCCCT CCACC GTTAA  
CAGCA CCGCA ACGGG AGTTA CCTCT GACTC TTATC AGAAT ACAAC AACTC AGCTG CCTGC ATCTT  
CTTCT GCCGC TGCC TAAAGT CTTCC ATCTG CGTCA GCGGT GCGAG CCAA TCTCC GAGCT CATTT  
TCAGA CACAT ACCCT ACCGC CACGG CCTTG TGCGG CACAC TGGTG GTGGT GGGCA TTGTG CTGTG  
CCTAA GTCTG GCCTC CACTG TTAGG AGCAA GGAGC TGCCG AGCGA CCATG AGCCG CTGGA GGCAT  
GGGAC CAGGG CTCGG ATGTG GAAGC TCCGC CGCTA CCGGA GAAGA GCCCA TGTCC GGAAC ACGTA  
CCCGA GATTC GCGTG GAGAT CCCAC GCTAT GTTTA ATAAA AACTG CGGGC ACGGG GGACG GCGTT  
GTTGT ATATG TGAAT TTGTA AATAA TAAAT GGGAC CCCAT CCTGT AAAAA TACAG AGTCC GTGTC

AGTCT CTGAA GGACA GAGTA TTGGC ATATA GCCAA TAGAG ATAGT TGTGG CAAAG AGCCA TGTTA  
TGGAT TAGTA ATGGA AAGTA TCGTC ACCAA TAGGG GAGTG GTCAA TAATG GTCAA TAACC CACAC  
CTATA GGCTA AGCTA TACCA TCACC TATAG CATAA GGAAG CGGGG GTGTA TAGGC CCCAA GCCAA  
AAACA GTATA GCATG CATAA GAGCC AAAGG GGTGT GCCTA TAGAG TCTAT AGGCG GTACT TACGT  
CACTC TTGGC ACGGG GAATC CGCGT TCCAA TGCAC CGTTC CCGGC CGCGG AGGCT GGATC GGTCC  
CGGTG TCTTC TATGG AGGTC AAAAC AGCGT GGATG GCGTC TCCAG GCGAT CTGAC GGT

**UL132 nucleotides 176380 to 177192:**

CTAGT CGTAC TCGGG ATCTC TGAGC GAGAC GGGTT GCATG GCAAC TTTCA TTAGT TTGGG AATCT  
GCCAG CTGGT GCTGT TCGAA GGTTT TTCCA TTTCC GAGGC GGTC AATTCA TCGTA CACCG AAACG  
TAGTA CCTGA TGGGG TCCTC CTCAT TGTCC GAGAG GTGAG ATTCC ATGGT CAAAG GCGAG CCTCT  
CCCAT AATTG GGATT CACGA ACGAC GTGTC CAAGT TGCCA TCCTT TCTGA AATAG ATGAC GTTCT  
CAGGA TCATG TTTCA TGCGC TCGCG GGCCG CGGAC GCCTC CTCTT CCTCG TCCCA GTCCC GAGTT  
TCCAA CCGCT GATAA GGGCT CGAGG AACAA AATCC GGCGG GGATC TGAGA ACCTC GTCGG GAACC  
GCTGC CAAAC GGGCT GCTGC CGCCA CTGTC GTCCG TGTCG TCCAA CAGGT TGACG GCCTC TTCGT  
CGGCG AAACG AAAGC GGCCC GGGTG CTTGC AACAC GAGGA GTAAA CTACC GCGAT CAGTA CCGCT  
ATGAA GCTGA AAATG GAGGT GCCTG TCACG ATGTA GAAGA GGATA GCCAG CACTT TCATG ATTTT  
GTCAT TGCGC GCGTC GTGAA CCGAA GATTC GCGGG CCGTG GTCAT GTTGG TTTTC GTTGT AGGTT  
CGCTA CTCGT GGTGC TCTCG ACGGT ATTTT TGCTG CTGGT GCTAG TAGGG ACGTT TGTGC TGCTG  
GTCAT ATTTT TAGCG TCGCT GAAGT CGATG TGAAG CAGCA ACCCG AACGC GACCA GGACC AGGAA  
TGTTG CCGCA AGGAG ACCCC GCGGG GCCGG CAT

**US17 nucleotides 198929 to 199312:**

TCATA GAGAC ACAAG GTGAG CAGGT CGGCC TCGGA CCACG CGATC TCAAA CAGGC GCGTG GTGTC  
AAAGA CCGTG ACGAC CAGCA TGAAG CTGAG CGCCA TGGCG TAATA GCCCA AAAAA AGTTT GTGCC  
CTAAC GGTAC GGGTT GCAGG TAAAG TGCGA TCAAG AACGC GATAA CGCCG ATCAC AAACA GCGTG  
ATGAT GACCT GCCAT CGACG GTGAT TATGG GCGGC TAGAC CCGTG ACGCA GCTGC AGAGG CTAAA  
AAGCA CGCAA GCCAA GAGGC CCGAG AAGGT CACCA GCGTA GAGGA GGAGC AGGCG CTGGC CACGA  
TCACC GAAAG CGTGC TGAGC ACGCT GTAAA TGGTG AGCAG GCCCG GGCTC GGCGG CGAC

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