

Simian varicella virus DNA is present and transcribed months after experimental infection of adult African green monkeys

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To study the pathogenesis of simian varicella virus (SVV) infection in its natural primate host, we inoculated adult SVV-seronegative African green monkeys intratracheally with 10^3-10^4 PFU of SVV, sacrificed them 11 days, 2, 5, 10, and 12 months postinfection (p.i.), and examined lung, liver, and ganglia for SVV DNA and RNA. PCR analysis revealed SVV DNA in ganglia and viscera at 11 days and 2, 5, and 10 months p.i. Similarly, SVV transcripts corresponding to immediate early (IE), putative early (E), and late (L) SVV open-reading frames (ORFs) were found in liver, lung, and ganglia of most monkeys at multiple intervals for the 12-month study period. SVV-specific antigens were detected in ganglia and liver during acute varicella, but not in ganglia 12 months p.i. Analysis of control tissue (ganglia, lung, and liver) from uninfected SVV-seronegative adult African green monkeys did not reveal SVV DNA, SVV RNA, SVV-specific antigen, or varicella-specific pathological changes. Overall, intratracheal inoculation of SVV in African green monkeys resulted in the presence of viral DNA and transcription of multiple viral genes in many tissues for months after experimental infection. Journal of NeuroVirology (2002) 8, 191-203.

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Introduction

Simian varicella virus (SVV) is an alphaherpesvirus that produces a naturally occurring exanthematous disease of nonhuman primates that mimics human varicella (Clarkson *et al*, 1967; Soike *et al*, 1984; Padovan and Cantrell, 1986). Further, like varicella zoster virus (VZV), SVV remains in ganglia at multiple levels of the neuraxis for the life of the host (Mahalingam *et al*, 1990, 1991). Months after primary infection, most, if not all, of the SVV genome is present (Mahalingam *et al*, 1992), and the SVV open reading frame (ORF) 21 is transcribed (Clarke *et al*, 1996).

The SVV and VZV genomes share considerable nucleotide sequence homology (Gray and Oakes, 1984). SVV ORFs corresponding to VZV immediate early (IE) genes 4, 62, and 63, which encode regulatory proteins (Felser *et al*, 1988; Jackers *et al*, 1992; Defechereux *et al*, 1993), have been identified (Clarke *et al*, 1993; Gray *et al*, 1995, 2001), as well as ORFs homologous to VZV genes 21, 28, and 29, which encode putative early (E) proteins (Kinchington *et al*, 1988; Bergen *et al*, 1990; Mahalingam *et al*, 1998). A homologue of VZV gene 40, which encodes the late (L) major viral capsid protein (Davison and Scott, 1986), is also present in SVV.

To study the pathogenesis of SVV in its natural primate host, we inoculated 12 adult African green monkeys intratracheally with SVV (Figure 1).

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Figure 1 Outline of protocol used to detect SVV DNA and SVV-specific transcripts in multiple tissues from adult African green monkeys inoculated intratracheally with 10^3-10^4 PFU of SVV.

To obtain as many monkeys as possible, we included three that had been treated with an experimental non-nucleoside antiviral drug; one monkey that had been treated with interferon- β before and during acute varicella; and four monkeys that had been treated with oral famciclovir daily for 7 days at 3 months and 4 months after experimental inoculation. Trigeminal ganglia (TG) and dorsal root ganglia (DRG) as well as lung and liver were analyzed at various times for viral DNA, RNA, and antigen.

Materials and methods

Cells and virus

African green monkey kidney (BSC-1) cells and SVVinfected BSC-1 cells (SVV-BSC-1) were propagated as described (White *et al*, 1997).

Experimental inoculation of African green monkeys Adult African green monkeys (M1–M12) were inoculated intratracheally with 10^3-10^4 PFU of SVV (Figure 1). Table 1 summarizes the inoculum dose, antiviral treatment, viremia, and severity in each monkey. Viremia developed 5 to 7 days p.i. and rash developed 7 to 11 days p.i. Monkey M1 and M2 died 10 and 11 days p.i., respectively. Monkeys M3– M12 were sacrificed 2 (M3–M5), 5 (M6–M8), 10 (M9– M11), or 12 (M12) months p.i. Monkeys M3–M12 did not have varicella or zoster when sacrificed. Animals M2–M5 received no antiviral treatment. Monkey M1 was treated with 5×10^3 IU/kg of interferon- β on days 1 and 6 p.i. Monkeys M6–M12 received 100 mg of oral famciclovir daily for 7 days at 3 and 4 months p.i. In addition, monkeys M6, M11, and M12 were given 20 mg or 40 mg of an experimental antiviral drug (Wyeth-Ayerst, Radnor, PA) twice daily for 10 days starting 48 h p.i. The experimental drug is a small hydrophobic non-nucleoside molecule with an approximate molecular weight of 500 Da. At the time of sacrifice, samples of lung, liver, and pooled TG and DRG from individual monkeys were quick-frozen in liquid nitrogen and stored at -70° C.

DNA and RNA extraction

Frozen viscera and ganglia were pulverized with a mortar and pestle in the presence of liquid nitrogen. DNA was extracted from tissues using a tissue kit (QIAGEN, Inc, Santa Clarita, CA), and total RNA was extracted using TRIzol (Life Technologies, Inc, Gaithersburg, MD). Since DRG from monkey M3 were attached to the spinal cord, the tissues were processed together. Poly(A⁺) RNA samples were prepared from total RNA extracted from lung and total RNA extracted from spinal cord/DRG from monkey M3 using an mRNA separator kit (Clontech, Palo Alto, CA).

In vitro transcription

The pSP64 Poly(A) vector containing the green fluorescent protein (GFP) gene was linearized using

 Table 1
 Viremia and rash in adult African green monkeys

 experimentally infected with SVV by intratracheal inoculation

Monkey	Sacrificed (time p.i.)	Inoculum (PFU)	Antiviral	Viremia ^f	<i>Rash</i> ⁱ
M1	10 days ^a	2.7×10^{3}	ves ^b	TNTC	4+
M2	11 days ^a	2.0×10^4	no	NA ^h	3+
M3	2 mo	2.0×10^{4}	no	NA	2+
M4	2 mo	1.5×10^{3}	no	13 PFU	1+
M5	2 mo	$1.5 imes 10^3$	no	9 PFU	2+
M6	5 mo	$4.0 imes 10^3$	ves ^c	660 PFU	3+
M7	5 mo	$4.0 imes 10^3$	ves^d	690 PFU	2 +
M8	5 mo	$4.0 imes 10^3$	ves^d	1550 PFU	2+
M9	10 mo	$4.0 imes 10^3$	ves^d	TNTC	2 +
M10	10 mo	$4.0 imes 10^3$	ves^d	285 PFU	3 +
M11	10 mo	$4.0 imes 10^3$	vesc	945 PFU	3+
M12	12 mo	$4.0 imes 10^3$	yes ^e	1550 PFU	2+

^aFound dead at indicated times.

 $^{b}5 \times 10^{3}$ IU/kg of interferon- β on days 1 and 6 p.i.

^c20 mg of an experimental antiviral drug twice daily for 10 days (started 48 h p.i.) and 100 mg of famciclovir daily for 7 days at 3 months and 4 months p.i.

^d 100 mg of famciclovir daily for 7 days at 3 months and 4 months p.i.

⁶40 mg of experimental antiviral drug twice daily for 10 days (started 48 h p.i.) and 100 mg of famciclovir daily for 7 days, at 3 months and 4 months p.i.

^fViremia determined by the detection of a varicella-specific cytopathic in Vero cells after cocultivation with blood mononuclear cells from day 7 p.i.

^gTNTC = plaques too numerous to count.

 $^{h}NA = not available.$

ⁱSeverity of rash determined by Dr. Kenneth Soike (Tulane Regional Primate Research Center) based on the extent of rash and hemorrhagic vesicles.

VspI restriction enzyme, and 400 ng of DNA were transcribed *in vitro* using SP6 RNA polymerase. RNA was cleaned using the OIAGEN RNA cleanup kit per the manufacturer's instructions and eluted using 50 μ l of RNase-free ddH₂O.

PCR amplification of actin and SVV DNA

Based on published SVV DNA sequences (Clarke et al, 1993, 1996; Fletcher and Gray, 1993) oligonucleotide primers were selected using DNASIS software (Hitachi Software Engineering America Ltd, South San Francisco, CA) (Table 2). Table 3 lists the primer sets used in the primary and nested PCR to amplify actin and SVV ORF 4-, 21-, and 63-specific sequences, and the sizes of nested PCR products. For the primary PCR, $100-\mu$ l reactions contained 0.1–0.5 μ g DNA or total RNA in 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μ M each of dATP, dCTP, dTTP, and dGTP, 1.5 mM or 3 mM MgCl₂, 0.2 μ M of each primer, and 2.5 units of Perkin-Elmer Taq DNA polymerase (Foster City, CA). Samples were overlaid with mineral oil, heated to 80°C for 5 min, and cycled 35 times in an automated DNA thermal cycler (Perkin-Elmer) programmed as follows: denaturation at 94°C for 30 s; annealing at 55°C for 30 s (60°C for SVV ORF 40 primer set); and polymerization at 72°C for 1 min, extended in the final cycle to 7 min. Nested

PCR conditions were the same as before, using 5 μ l of a 1:20 dilution of the primary reaction product.

RT-PCR amplification of GFP-, actin-,

and SVV-specific transcripts

GFP, actin, and SVV ORF 4, 21, 28, 29, 40, 62, and 63 transcripts were detected by nested RT-PCR followed by Southern blot hybridization. Total RNA extracted from lung, liver, and ganglia and GFP in vitro transcription reaction products were treated with RQ1 RNase-free DNase (Promega, Madison, WI) until no amplifiable GFP or SVV DNA was detected. To determine the sensitivity of the RT-PCR reaction, 3 μ g of DNased total ganglionic RNA was mixed with 1, 10, 100, 1000, or 10000 copies of GFP *in vitro* transcripts before incubation with AMV reverse transcriptase (+RT) (Promega). For all other reactions, 3 μ g of DNase-treated total RNA or 2 μ g of poly(A)⁺-selected RNA (M3) was incubated with AMV RT (Promega). Negative controls were provided by omitting RT from the reaction. cDNA synthesis for GFP was primed using the oligonucleotide primer LU-T7-oligo(dT) (Table 2), and all other cDNA synthesis was primed using the oligonucleotide primer RACE-oligo(dT) (Table 2). DNase-treated total RNA (3 μ g) or poly(A)⁺selected RNA (2 μ g) was mixed with 0.3 μ g RACEoligo(dT) and double-distilled (dd) H₂O to a volume of 17 μ l, and heated to 65°C for 5 min. After cooling the mixture to 43°C, 2 μ l of 10 mM dNTPs, 0.5 μ l of bovine serum albumin (10 mg/ml), 5 μ l of 5 × RT reaction buffer (Promega), and 10 units of AMV RT or nuclease-free H₂O were added to a final volume of 25 μ l. Reactions were incubated at 43°C for 2 h, and RT was inactivated by incubating samples at 95°C for 5 min.

After reverse transcription, nuclease-free H_2O (75 μ l) was added to each RT reaction to a final volume of 100 μ l. For each primary PCR, 5–10 μ l of the RT product was used to amplify GFP, actin, and SVV ORF 4-, 21-, 28-, 29-, 40-, 62-, and 63-specific cDNAs. Primary and nested PCRs were performed as described previously. The sequences for the GFP, actin and SVV ORF 4-, 21-, 28-, 29-, 40-, 62-, and 63-specific primers are shown in Table 2. The actin RT-PCR was not nested. Advantage-GC 2 polymerase (Clontech) was used to amplify SVV ORF 62 cDNAs. The primary SVV ORF 62 PCR reaction included 5-10 μ l of diluted RT sample, 10 μ l 5× GC 2 PCR buffer, 10 μ l GC-Melt, 2 μ l 10 mM dNTPs, 2 μ l of each primer (5 μ M stock), 1 μ l 50× Advantage-GC 2 polymerase mix, and ddH₂O in a final volume of 50 μ l. Samples were overlaid with mineral oil, heated to 95°C for 1 min, and cycled 35 times in an automated DNA thermal cycler (Perkin-Elmer) programmed as follows: denaturation at 94°C for 30 s; annealing at 55°C for 30 s; polymerization at 68°C for 1 min, extended in the final cycle to 7 min. Actin amplification was performed on cDNA reverse-transcribed from lung and liver RNA.

 Table 2
 Oligonucleotides used in this study

ORF 44P1ACTGGAATCTGCAGAGGAA4639°4P2TGATGGTGTCTCTCAGCAGACAA41764P3GTCTTTAACCGCCTCATG41974P3TAATACACCGCCTTAACAGA4281Phi4TAATACACCGCCTTAACAGA4281Phi4TAATACACCGCCTTACAGAA3168621P2CGACGCTCTGTTACAACGAAAGTCGT3198521P3AAGCAGOGCAATGGTAAAAAGTCGT3198521P4TGCACCCTCTGTTACAATCG3198121P5AAGCAGOCGCATGGTAACAAAGTCGT3198121P5AAGCAGCCCTCTGTTACAATCG3198121P5AAGCAGCGCCTCTGTTACAATCGC3198121P6AAGCAGCCCCCTCTGTTACAATCGC3198121P7AAGCAGCGCCGCTCTGTACAAACAGGTG3193021P8TAACATTCAGCGATGCCAA34222PA121BTAACATTCACGGATGTCCAA34222PA228CGACCTCTCTCTCTCCCATACAGCGG3450021P7AACCGTTCCCCATACATCGCGACT3422PA28CCAACCCAGCAGAGTGTCCAA3422PA29CGACCTCTCTCTCCTCTCCCATAC3450028P3CCAACCCAGGAGTGTCCAAA342228P4ACCCCTCTCAAGCAACGCGACT3450028P3CCAACCCAGCAGAATGTAAC3452028P4ACCCCTCTCAAGCAGCAGCAATGTAC3455028P3CCAACCCAGCAGCAACACC3455028P4CCCTTACAGCAGCGCTCTCTCTCTCTCTCT3465028P3CCAACCCAGCCCAACCACCACC3565028P4CCCTTTACAGCGCTCTCTCTCTCTCTCTCT3465028P5CCCAACCCAGCCGCAACACCACCCCCAACA3552929P4CTACTAGCGCTCCCAACCCCCCACCACCACCCCCCAC	Gene	Name	Sequence (5' to 3')	Location
4P2 TGATGGTCTGTGTCAGGAGAA 4176 4P3 GTCGTGTGATAGGCGCTGATG 4497 4P4 TAATACACCGGCCTTACAGAA 4265 Phb4 TTATCCCGCTAACGCAAACAG 4265 21P2 GTCGCTGAAACACAGC 31667 21P3 AAGCACGGGAATGGTAGCACGTAAACAGC 31897 21P4 TGCACGTAAAACAATCGC 31897 21P4 GCACACTCACGGGAATGGTAACTTGC 31891 21P4 GCACACTCACGGGAATGGATAA 34079 21P5 ATGCGGCGGAATGGATAA 34079 21P6 GTACATCUCAGTAACACTTGC 34390 21P7 AAGGTTCGCGAATGGATAA 34222 Pho21B TAACATTCGCGGATGGATAA 34222 Pho21B TAACATTCGCGAATGGATAA 34222 CRF 28 28P1 TAACGTCGTCGCACAGCGA 34222 28P3 CGACTGTGTGTGCGCAGTGCAA 48476 28P3 CGACTGTGTTAAGGCCATTGC 48765 28P3 CGACTTGTGTGTGCGCAGTGCA 48765 28P3 CGACTTGTGTGTGCACGCAGTGA 48765 28P3 CGACTTGTGTGTGTGCACGCATGGA 48765 28P3 CGACTTGTGTGTGTGCACGCATGGA 48765 28P3 CGACTTGTGTGTGTGCACGCATGGA 48765 28P3 CGACTTGTGTGTGTGAACTGCCATGGA 48765	ORF 4	4P1	ACTGGAATCTGCAGACGAA	4639ª
HP3CTCTCTTCATACCGCGTCAATG44974P4TAATACACGCGCCTTCACAAGA4265Pb4TTATCCACCGCCTAAAACAG426521P2CTCGCCTAAAACAGCACCGCACTCAGA3196521P3AAGCACGGCAATCGACACATCGCA3195721P4TCGCACCTGGCTAAACATCGCACAACTCCTG3198521P3AAGCACGGCAATCGACAACTCCTG3198521P4TCGCACCTGTGAAAATCGCACACTCCTG3439021P5ATCGCCCTGGCAACATCGCACACATCGC3439021P6CTACATCCACGGCTTTCGCACAACTCC3439021P7AACCTTCCCCATACATCAGCGTG34422221P6CTACATCCCCCATCACATCGC3439021P7AACCTTCCCCATACATCAGCGTG34422220R723P6CTACATCCACGATGCCAA3422220R723P1TAATCGATCTCTCCCCACACATCC3439220R723P2CCAACCAACGACCTTCCCCACACA3422220R723P3CCAACCAACGACCTTCCCCACA3422220R723P4ACCCCTCTCACATCGCACA3422220R723P3CCAACCAACGACCATTCCCACACACA3491020P4CCTATACACACGCCCTCCCCCCTCTTCTCTCTCT3493620R729P3CCATCACCACCCCCCCCACATCA3471020P4CCTATACACACGCCCCTCCCTCTTCTCTCTCTCTCTCTCT		4P2	TGATGGTGTGTCTCAGGAACA	4176
Ph4 TAATACACCGCCCTTACAAGA 4265 Prb4 TTATCCCCACGTAAAACAACAG 4346 ORF 21 21P1 CCCACTTCCCCTAAACTACAACGA 4346 21P3 AAGACAGGGAATGGTAGAAACTCGCT 31957 21P4 TCCACGCGTGTATAGAAATCGC 31851 21P4 TCCACGCGGTGTGTAGAAACATCGC 31950 21P5 ATCCCCGCGGGATCGAATAACGCTACA 34470 21P6 GTACATCCCAGTACATTCC 34390 21P6 ATCCCCACTCCACGTCAAACAACTCC 34390 21P6 ATCCCCCGTGGAATCGCACACGCGT 34322 Pb21B TAACATTCCCCATCACAGGTC 34322 Pb21B TAACATTCCCCCACTTCCCACCAC 34222 ORF 28 28P1 TAACATCTCTCCTCCCCACTTCCACACA 48476 28P2 CGACTTGTTCTCTCTCTCTCTCT 49619 ORF 29 29P1 CTATCACGCACTGCAACGACACCACCACGACG 48019 ORF 29 29P2 CTATCACGCACTCCATCC 54950 29P4 CCCTTAACTCTCCACCTCCATCC 54950 29P2 CCTATACCACCACCACACACACACACCACC 5529 ORF 61 40P1 CTACCTCCACCTCCATCCC 5529 ORF 62 62P1 CCTTACACTCACACCACCACCC 5529 ORF 62 62P2 CCACCCCCCCTTACACTCCCATCCC 5529 ORF 63		4P3	GTCTGTTGATACGGCGTGATG	4497
Phb TIATCCCCACGTAAAACAG 4346 ORF 21 21P1 CCCACTTCGCTAAAACAGCACTTGGA 31686 21P2 GTGCGTAAAAGCATCGCT 31785 21P3 AAGCACGGGAATGGACAAAGTCGTG 31896 Phb21A GCCACCTGTGTAGCAAAGTCGTG 31896 21P5 ATGCGCGTGGAATCGACAACTCG 31891 21P6 GTACATCCACGGGTTGCACAA 34079 21P6 GTACATCCACGGATGGAACATCGC 34390 21P7 AACGTTCCGCATACATCAGCGTG 34390 21P8 ATTGCGTCACAACAGCCATTCC 34390 21P8 TAATCGATTCATCGACAAGCCATTCC 34322 ORF 28 28P1 TAATCGATTCGTCCACAAGCAGTTGCACA 48222 ORF 28 28P3 CCACTCAACAAGCATTGCACA 48222 ORF 29 29P1 CTACGGTTATACGGACTTGCACA 48360 ORF 29 29P1 CTACGGTATACACGACTATGTGTGTCT 54650 29P3 CCATTACAGCGTTTGCACTGCAATGC 54795 29P4 CATTTACTGGACACAACAATATTA 54710 29P3 CGCTTACAGACGCTCCTCACTCGT 54850 29P4 CATTACTGCACCACAACA 54795 29P3 CGCTTACAGACGCACACCA 54795 29P4 CATTACAGCGCTACTCCACTCGT 54850 00F4 40P1 CTACCACGCCACACACA		4P4	TAATACACCGGCCTTACAAGA	4265
ORF 21 21P1 CCCACTTCGCCTAAACTTCGA 31666 21P2 GTGGCGTAAAGAATGCTACGCT 31957 21P3 AAGACAGGGAATGGTAGAAAGAATGCT 31785 21P4 TCCACCGCGTGTATAGAAATGC 31890 21P5 ATCCCACCGTGGTATAGCAAATGC 34079 21P6 GTACATCCCAGGTACAATTCC 34390 21P6 ATCCCACGTGCAATGCACACGCT 34150 21P6 GTACATCCCAGTACATTCC 34390 21P7 AAGGTTTCCGATACATCACGGTGT 34422 Pb21B TAACATTCCCGATGCACACGCT 34422 Pb21B TAACATTCCCCGATGTCCAGCA 44476 28P2 CGAATCTACGCATGTGCGAAT 44876 28P3 CGAACCAACGAAGCTGAAATGGA 48181 28P4 CGATTTACTGTCTCTCTCTGTGTT 49051 28P3 CGCATACGAACGCAAGCAAGCAGCAAGCAGCATATTA 49161 28P4 CGCTTACATCGCATGG 48019 28P2 CGCATACACAAGCAAGCTCCATGG 54950 29P3 CGCTTACGCCCTTAACCACTCGCT 54950 29P4 CGCTTACGACGCACATCACA 54950 29P4 CGCTTACACGCCCTTGCACCACACA 54950 29P4 CGCTTACACGCCCCATGCC 5529 20R4 CGCTTACACGCCCCATACA 75228 20R5 62P1 CTACCCCCCGCATACACACC		Prb4	TTATCCCCACGTAAAACAG	4346
21P2 CTGCGCTAAAAGAATCGCT 31957 21P3 AAGACACGGCAATCGACGAACTCGTG 31785 21P4 TGCACGCGTGTTAGAATTCG 31891 21P5 ATGCCACTCACGCGTTTGCA 34079 21P6 GTACATCACGCGTTTCCA 34390 21P7 AACGTTCCCGATACACTCACCGCT 34390 21P7 AACGTTTCCCGATACATCAGCTC 34320 21P8 ATTCGTCTCCATACACACGCTC 34322 Pb21B TAACATTCACGCGATCCACAC 46476 28P2 CCACTTGTTCCTCCTCGTAATG 46019 28P3 CCAACCTAGCGCGCACATTGG 46019 0RF 28 28P1 CTATCAAGGTCGCCAACT 46019 28P2 CATTATACTGCGAACTGCCAAC 46019 0RF 29 29P2 GTATCAAGGTCGCCACTGCCAACT 46019 28P4 CGTTACAAGCTCCCAAC 46019 28P4 CGTATCAAGCTCCCAACA 47953 28P4 CGTATCAAGCTCCCAACA 4795 28P4 CGTATCAAGCTCCAACA 54936 29P2 GTATCAAGCTCCAACACA 5728 40P3 CTAACTACACGCGTTTACAACA 57528 40P3 CTAACTACACACACGCGTACAC 75728 40P3 CTAACTACACACACGCGTCCCAACA 75529 00F62 62P1 CTATCACACACACGCGTCCCACACA 7552	ORF 21	21P1	CGCACTTCGGCTAAACTTGGA	31666
21P3 AAGACAGGAATGCTAGAAAGTCCTG 31785 21P4 TGCACGCGTGTTAGCAATTCG 31896 21P5 ATGCGCGTGGTATCGAATTCG 34079 21P5 ATGCGCGTGGATACACTCAGCGTCG 34150 21P6 GTACATCCCAGTACATTCC 344390 21P7 AAGTTCAGGATCAACGAGTG 34150 21P8 ATTTCGTCTATCATAAGCCATCA 34222 Ph21B TAACATTCACGAGTGCAA 34222 ORF 28 28P1 TAACATTCAGGAGTGCAA 48476 28P2 CGACTTCTCGTCACATC 4867 28P3 CCAACCAACGAAGCAACGAAGCTGCAA 48181 28P4 AGCCTCTGTGAATG 4869 28P3 CCAACGAAGCAAGCAAGCTGCAATTAG 48181 28P4 CGTTAACGTCATGCAAGG 48019 ORF 29 29P1 CTATCAGGAACTGCATTGG 54550 29P3 CGCTTACAGAGCAGCAGCAATTTA 54795 29P3 CGCTTACAGAGCAGCACAATTTA 54795 29P4 CGTTACAGAGCAGCCACATTAG 7528 40P1 CTATCAGGAAACCC 7528 40P2 TTGACTTAGCAGCAGCACACA 7529 QFF 40 4091 CTATACGTCAACCCC 7528 40P3 CCTATACAGCAGCACCCGTACA 7528 40P4 CGAGTAGTCACACCCCCTCCCT 10459 <		21P2	GTGGCGTAAAAGAATCGCT	31957
21P4TICACGCTGGTTAGAATTCG31896121P5ATGCGCGTGGAATGGATAA3407921P5GTACACCATCCCGATACATCG3439021P6GTACATCCCCAGTACATTCC3439021P7AACGTTTCCGATACATCAGGTG3432221P8ATTCGTCTATCATAAGCCATCT3432221P8TAACATTCACGGATGCCAA3422221P3CGACTTGTCGCAGTGCCAA3422221P3CGACTTGTTCCTGCGAGTGCCAGA4801628P2CGACTTGTTCCTGCTGTGTGTC4796528P3CCAACCAACGAAGTGCAAA4818128P4CGACTCGTAGCATTGGAA4801928P4CGACTCGTAGCTGCGCGCTGTGTGTC5465029P3CGCTTACCGGACTGCTGCGCGATTCCTTGTGT5493629P4CGTTACAGGTGGTCGCTCGTGATTCTTCTGTGT5493629P3CGCTTACCGGACTGCGACGACAATTTTA5471029P4CGTTATACAGGACAGCAGCA5479529P3CGTTACAGAGCAGCACAC5479529P4CGTATCAGAGCAGCACACC552829P3CGTTACAGACGACGACACC7518840P2TIGACTTACGAGCAGCACACC7552840P3CCTATACAGACAGCGCTACC755290RF 6262P1CGACCTGCCCGCTTATAGC10632162P3ATGCGGCTATGCTCCTCGCCCCCT10434162P4GGACGTCGATGTCCTCGCCCCCC1045964P4GGACGCGCATACCCCCCCCCCCAAA11032263P3ACCCGGCCATATCCTCGCCCCCCCCCCAAA11032264P4GCACCCCCCCCGATTAACCCCCCCCCCCCAAA11042964P4GCACCCCCCCCCTATGCTCTCCCCCCCCCCCAAA11042964P4GCACCCCCCCCCTATGCTCCCCC		21P3	AAGACAGGGAATGGTAGAAAGTGCTG	31785
Ph21A GACACATCAGCGGTTTGCA 31821 21P5 ATGCGCGTGGAATAA 34079 21P6 GTACATCCCAGTACATTCC 34390 21P6 ATCTTCGCTTACAACATCAGCGTG 34150 21P8 ATTCGTCTATCATACACTCAGCGACTTC 34222 Ph21B TAACATTCACGAGTACATTCAGCACACA 34222 ORF 28 28P1 TAATGATCTTCTCCCACATTCCACACA 48476 28P3 CCAACCAACGAAGCTGCAA 48181 28P4 ACCCTCTGTAAGTCTTGTGTCC 47951 28P3 CCAACCAACGAAGCTGCAAATAGA 48181 28P4 ACCCTCTGTAAGTCTTGTGTC 49951 28P3 CCAACCAACGACGACGAATTGC 48019 29P3 CTATCAGGTAATGCGCGTATTGC 49936 29P3 CCTACGAGACGACGACAATATTA 54710 29P3 CCTATCAGACGCAGCAACACCA 54850 29P4 CGTATCAGCGGTTTTAGACC 7528 40P3 CCTATCAGACGACGACACA 7528 40P3 CCTATCAGCACGACCACACCA 7528 40P3 CCTATCAGCACGACGACACA 7528 40P3 CCTATCAGCACGACGACACA 7528 40P3 CCTATCAGCACGACGACACA 7528 40P4 TGACCTCCCCCGCCTTAACC 7528 40P3 CCTATCAGCACGACGACACA 7528		21P4	TGCACGCTGTGTTAGAATTCG	31896
21P5 ATGCGCCTGGAATGGATAA 34079 21P6 GTACATTCC 34390 21P7 AACGTTTCCGATACATCAGGTA 34320 21P8 ATTGCGCATCACATCAGGACTGT 34320 Ph21B TAACGATTCAGCGATGTCAA 34222 28P2 CGACGTCTCTCCCCCGCACTTC 48476 28P3 CCAACGAAGCAACGACGCAATTC 47805 28P4 ACGCTCTGTAGTCGCACTTCCAGCA 47805 28P3 CCAACGAAGCAGCACGCAAATAGA 48019 0RF 28 29P4 CACGCTCTGTAGTCGCAATTGC 47805 28P4 ACGCTCTGTAGTCGCAATTGG 4650 28P3 CCATTACAGCAGCAACTGCAAGC 48019 0RF 29 29P1 CTACGACGCACTGCAATGG 48019 0RF 29 29P3 CGTTACAACGCGCATTTGG 54550 29P4 CGTATCACAGCAGCAAATTTA 54710 29P3 CGTTACTACAGCAGCAAAC 75728 29P4 CGTATCACAGCAGCACAACC 75829 0RF 40 40P1 CTAACGCGTCGCCACTCACC 75801 40P2 TTGACTTACAGCAGCACAACA 75622 62P4 CCACCCCGATAAACCGCCTCTAAC 75829 0RF 63 63P1 CCACCACGCAGTAACACC 75829 0RF 63 63P1 CCACCCCCGATAAACTCTCT 104341 6		Prb21A	GACACATCAGCGGTTTGCA	31821
21P6GTACATCCACGTACATTCC3439021P7AACGTTTCCGATACATCAGCGTG3415021P8ATTCGTCTATCATACACACGGTG34222Ph21BTAACATTCACGATGTCCAA34222ORF 2828P1TAATGATGTCTCCCACTTCCACACA4847628P2CGACTTGTTCCTCCTCGTAATG4796528P3CCAACCAACGAAGCTGAAATAGA4818128P4ACGCTCTGTAAGTCTTGTGTC4796528P3CCAACCAACGAAGCAAGCAACGAAG48019ORF 2929P1CTACGGTTATACCTGCAAGTCCTCTCTCTGT5493629P2CTATGAGACTGCCAAG5475029P3CCATCTACAGACGACGAATATTAC5471029P3GCTTACAGACGCTACTGCT5485029P4CGTTACAGACGCTACC557850RF 4040P1GTACCTTACAGACGACGACAACA751880RF 4040P2TGACCTTACAGACGCGCTACC7530140P3CCTATACAGACAGCGCTACC7552940P4AGACCTACTCCACCCGCTAG756220RF 6262P1CTACCTTCCACCGCTACA755290RF 6363P1CCACCCCCGGTTAAACTCT1045962P3CTACCCCCGATTCCACCCCCCCATACA10512162P4CAGTGTTCCAACCCCTCTAAC1045963P3ACCCCCCCCCAATTCTCCAACCCCCCCAA11052263P3ACCCCCCCCCAATTCCCCAACCCCCCAA131*064P4GACGTCTCTCTCCAACCCCCCAAA131*064P4GACGTCTCTCTCTCCCCAACCCCCAAA11052263P3ACCCCCCCCAATTCCCCCACCAACACCCCCAAA131*064P4GACGCCTCTCCTCTCCCCAACCCCCCAAAA11052263P3ACCCCCCCCCAATTCC		21P5	ATGCGCGTGGAATGGATAA	34079
21P7 AACGTTICCGATACATCAGGTG 34150 21P8 ATTICGTCATACACCATCT 34322 0RF 28 28P1 TAACATTCACGGATGTCCAA 34222 0RF 28 28P1 TAACATTCACGGATGTCCAC 4846 28P2 CGACTTGTTCCTCCTCGTCACGCAC 48476 28P3 CGACCACGACACGCAAATAGA 48181 28P4 ACGCTCTGTAAGCTCGATAGC 49019 0RF 28 28P3 CATTTACTGGGAACTGCATGGTCCTTCTGT 54936 28P4 CACGTTACAGACGCGAATTGGTCCTTCTGT 54936 29P2 CATTTACTGGGAACTGCACATCAC 54915 29P3 CCTTACAGACGACGAATATTA 54710 29P4 CGTTAACAGACGACGAAATTTA 54710 29P3 CGTTACAGACGACGTACAC 75728 29P4 CGTACTTACGACACGCCATACA 75728 29P3 CGTATACAGACAGCGCACATCA 75728 29P4 CGACCTACGTCACACCACACA 75728 29P4 CCATTTACAGACAGGCCACATACA 75728 29P4 CCATTACAGACAGCGCACATACA 75728 29P4 CCATTCACAGACAGCGCACATACA 75728 29P4 CCATTCACAGACAGCGCACATACA 75728 29P4 CCATTCACAGACAGCGCACATACA 7528 29P4 CCATTCACAGACAGCGCACATACA 7528 29P4 <td< td=""><td></td><td>21P6</td><td>GTACATCCCAGTACATTCC</td><td>34390</td></td<>		21P6	GTACATCCCAGTACATTCC	34390
21P8ATTTCGTCTATCATAAGCCATCT33222Ph21BTAACATTCACGCAGATGTCCAA3422228P1TAATGATGTCTCCCCACAA3422228P2CGACTCTTCTCCTCCTAATG4780528P3CCAACCAACGAAGCTGAAATAGA4818128P4CACTTCTTCCTCTGTAATG4795128P3CAACGACTGCAAG480190RF 2929P1CTACGGTTATACCTCGATGCGAGACTGCAAG4801928P2CATGCAGCTGCTGGACTGCTCTGCTGCTGGATGCTCTCTTCTG5493628P2CGCTTACAGCGCGCGCGACAATATTTA5471029P3GCCTTAATACCTCGCTAAC5485028P4CGTTAATACTCTCGCTAAC5485028P4CGTTAATACTCTCGCTAAC5485028P4CGTACAGACGACGACAACACC7518840P2TGACTCACCACGCCTAAC755290RF 4040P1CTAATCACACAGCGCTACC7530140P2TTGACTTACAGACAGCGCTACC755290RF 6262P1CTACACTCCCCACGCTAG10512140P3CCTATACAGACAGCGCTCCTCCTCCTCT10464562P4CACCCCCCCGATTAAACTCT10453162P3ATCGCATGCTCCACGTCTCCTCTCT10464562P4GACGTCTCCCACGTCTAACC11032263P3ATCCTACGCGCCATAGACACCCA11052263P4GACGCCTCTCTCCTCCTCCCT1045964P4GACGCCCCCCACGCCACACCCCACACA11052263P3ATCCCACCCCCACGCCACACCCCCACACA11037364P4GACGCCTCTCCTCTCTCCTCCT10464564P2CTACACCCCCCACACCCCCACACACCCCCACACA11037364P4GACGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		21P7	AACGTTTCCGATACATCAGGTG	34150
Phb21BTAACATTCACGGATGTCCAA34222ORF 2828P1TAACATTCACGGATGTCCAAG4447628P2CGACTTGTTCCTCCTGGTAATG4780528P3CCAACCAACGACGACGAAATAGA4818128P4ACGCTTGTAAGTCTTGTCT4795128P3CCATTTACTGGGAACTCGAAG4801928P4CACGTATCACGTCATTGG5465029P2GTATGAAGGTGGTGCGTGCATTCTTCTGT5493629P3GCCTTAACACGCACGAATATTA5471029P4CGTTAATACTGTACGTCCATCGT5485029P3GCCTTACAGACGACGAATATTA5475029P4CGTTAATACTGTACGTCACATCGT5485029P3GCCTTACAGACACGACAAATATTA5475029P4CGTATACAGACCACGCAATATTA5475029P4CGTATACAGACCACGCCAAAC751284002TTGACTTACAGACGGCTAAC75284003CCTATACAGACCAGGCTAAC75284003CCTATACAGACCAGGCTAACC75284004AGGCTAAGTCACTCGCACCATCA75284075CCAACCCCCGATAAACTCT10436162P4GCACCACCCCGATAACTCTCGCACCATAC10512162P3ATGCACCCCCGATTAACTCT10449663P4GCACACCCCCGATGAACCACCACCA11037363P4ATCTTACGCGCCTAATCCCCTCT10449663P2TTGCTACACCCCCCCCAACCACCACCCCCCCACAA11057263P4ATCTTACGCGCCCCAAGCACCACCCCCCCCAACAA11047363P4ACCCCGGATAGTCCAACCCCCCCCCCACAA11047363P4ACCCCCCCCCCCCCCCCCCAACCCCCCCCCCACAA11047363P4ACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		21P8	ATTTCGTCTATCATAAGCCATCT	34322
ORF 28 28P1 TAATGATGTCTCCCAGTTCCCAGTTCCAATG 48476 28P2 CGACTGTTTCTCCTCTCTCAATG 47805 28P3 CCAACCAACGAAGCTCAATGA 48181 28P4 ACGCTCTTACTGCAACGA 48019 ORF 29 29P1 CTACGGTTATACCTCCATCGTTCTCTCTTTTTT 54936 29P2 CTATCAAGCACGACGCAACGAACTCCTTTTTTTTTACATCTCCTTTTTTTT		Prb21B	TAACATTCACGGATGTCCAA	34222
28P2 CGACTIGTTCCTCCTCGTAATG 47805 28P3 CGACACCAACGAGAGGAAATAGA 48181 28P4 ACCCTCGTAAGTCTTCTGTC 47911 Ph28 CATTAACTGGAACTGCAACGACAGAATAGA 48019 0RF 29 29P1 CTACGGTTATAGTCTTGTGT 54936 29P3 GCTTACAGACGACGAATTTA 54710 29P3 GCTTACAGACGTCGTGCTCCATCCT 54850 29P3 CGTTACAGACGCCGTAATTTA 54710 29P4 CGTTAACAGCACGAAATATTA 54750 29P4 CGTTAACAGCACGACAAATTTA 54750 0RF 40 40P1 GTACTTACAGACACGCCACAAC 75188 40P2 TTGACTTACAGACAGCACAAC 75623 40P3 CCATATCACACAGCGCACAACA 75529 0RF 62 62P1 CTATCATCTCCCCACCGGTAG 75623 62P2 CCACCCCCGCATAACCCTCGACTTAAC 75529 0RF 63 63P1 CGACACCACCGGCTAACCCTCGTACACA 10434 62P2 CCACCCCCGCGTAACCCTCGTACT 104496 0RF 63 63P1 GCACACCACCGATGACGCACCA 110522 63P2 TTGCTCACACGCGCCCAATCTCTAACTCCGT 104529 64P4 ACCCCGCATGACCACCCCCCCCCCTAACCCC 10453 65P4 GCACACCCCCCACGCATACTCTTAACTCCT 104529 67P 63P3	ORF 28	28P1	TAATGATGTGTCGCAGTTGCAGCA	48476
28P3 CCAACCAACGAAGCTGAAATAGA 48181 28P4 ACGCTCTGTAAGTCTTCGTC 49951 Prb28 CATTTACTCGGAACTGCAAG 48019 ORF 29 29P1 CTATGAAGCGTGTAGGCATTGG 54936 29P2 CTATGAAGCGTGTCGCGTGATTCTCTTCTGT 54936 29P3 CCCTTACAGAGAGCAGAATATTA 54710 29P4 CCTTAATACTCTAGCTCCATCGT 54850 29P4 CCTTAATACTCTAGCTCCATCGT 54850 0RF 40 40P1 CTACTACAGACGACAAC 75188 40P2 TTGACTTACAGACAGCCACATACA 7528 40P3 CCTATACAGACAGCCACATACA 75622 40P4 CAGCTTAACTCCCCCCGCGATCTAAC 75623 40P4 CCACCTCCCGCACATACA 75629 62P1 CTACCTCCCACCGTGGATCTAAC 75629 62P3 ATGGACTCACCGCGGAGCTTATTG 104541 62P4 CACCTCCCCCCCGCGCCCCCCCCCCCCCCCCCCCCCCCC		28P2	CGACTTGTTCCTCCTCGTAATG	47805
28P4AGGCTCTGTAAGTCTTGTGTC47951Pb28CATTTACTGGAACTGCAAG48019ORF 2929P1CTACGGTTATACGTGCATGG5465029P2GTATGAAGGTGGTGCGTGCATTCTTCTGTGT5493629P3GCCTTACAGCAGCAGAATATTTA5471029P4CGTTAATTACTGTAGCTCCATCGT5485040P2TGACGCGTTATAGACGCGAAC5752840P2TGACTTAGAACTGCGTAAC7572840P3CCATACACACAGCGCACACAC755290RF 4040P4AGAGCTACTCGCACCGCCACC755290RF 5262P1CTATCATCTCCCACCGGTAC755290RF 6262P1CTATCATTCCCACCGGTAGC1043162P3ATGGACTTCCCACCGCGTATCT10445962P4CACGCCCCCATTAAACTCT10456662P4CACGCGCCCATTACACACGCGCTACT1045963P3ACCCGCATGACGACGCCCCCTTATT1045964P4ACGCGCTATCCCACCCGCTATAT1045965P2CTGGCTTATCCACCGCGCTATTT1045965P3ACCCGCATGACGACGCCCCCCTTATT1045965P4CTGCTCTACAGACTCCCGTACCCGT11052265P3ACCCGCATGACGACGCCCCCCGAA11037365P4TGACGACGCCTCTCTGCTGTGCAACCGCCCCCGAA1037365P4TGACCACGCCCCCCCACACCGCCAA1045967P-P1GACCCGCTCTCTTGCTGTGTCACCGCCCCCGCAA1045967P-P2TGCCCGCTCTCTTGCTGCTCGCCCCCCGCAA33067P-P3TGCTCACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		28P3	CCAACCAACGAAGCTGAA ATAGA	48181
Phb28CATTACTCGGAACTCGCATGG40019ORF 2929P1CTACGGTTATACCTGCCATTGG54565029P2GTATGAACGTGGTGCGTGATTCTCTTCTGT5471029P3GCCTTACACACGACGAATATTTA5471029P4CCTTATACTTAGCTCCCTCGT548500RF 4040P1GTACTCTTAGAAG547950RF 4040P1GTACTACAGACGACAAC7512840P2TGACCTACAGACGACAAC7522640P3CCTATACAGACAGCCTACC7530140P4AGACCTACTCGCACTGCACATCAC755290RF 6262P1CTATCATTCTCCCACCGGTACA755290RF 6262P2CCACCCCCCGATTAAACCTCT10451662P3ATGGACTGGATGTCCTCGTCCTCCT10466662P4GACTCTCTCAACCTCTCCTCT10451662P3ATCGACGGGATGCCCCGCATTAAT1042263P2TTGTCTACAGCCCACTACC11032263P3ACCCGGATGAACGTCTTGCACACAT11032263P4ATCTTACGCGCCACAA11032263P2TTGTCTACAGCGCCACACA11042764P4ACCTGCGTTCTACCCGTCCTCTCT10447665P2GTGCCTTATGCCACCACCACCA11032263P3ACCCGGATCATCTCGTAACCCGCTAAT11042764P4ATCTTACGCGCCACAACACCGCACA11042764P4ATCTTACCGCCCATGTCCCTCCTCAAACCCGC1043765P2GTACCACGCGTTCTCTCTCCGTAACTCCGAA11042764P4ATCTTACCACCCCCACACACCGCCAAA11042765P4ATCTACACCCCCCACGCACACACCCCCCCACAA11042764P4GACCACCCCCACTTCTCCCCCCCC24664P4ATCTACCCCCCCACGTCTCTCC		28P4	ACGCTCTGTAAGTCTTGTGTC	47951
ORF 29 29P1 CTACGGTTATACGTGCATTGG 54650 29P2 GTATCAAGCGTGCTCATTTGTTTTTGT 54936 29P3 GCCTTACAGACGACGACATATTTA 54910 29P4 CGTTAATTACTGTAGCTCCATCGT 54850 Ph29 TGATCCAGACGACGACGACGACGACGACGACGACGACGACGACG		Prb28	CATTTACTGGGAACTGCAAG	48019
29P2CTATGAAGGTGGTCGTGGTCGTGATTCTCTTGT5493629P3GCCTTACAGACGACGAATATTTA5471029P4CGTTAATTACTGTAGCTCCATCGT54850Ph29TGATCCCGTTTAGAACTCGGTAAC5718840P1GTACTTAGAACTCGGTAAC7518840P2TTGACTTACAGACAGGACAACA752840P3CCTATACAGACAGGCCTCCTGGATCAC752840P4AGAGCTAAGTCACTCGCACACACACA755290RF 6262P1CTATCATTCCCCACCGGTAG10612162P2CCACCCCCCATTAAACTCAT10434162P3ATGGAGTGGATGTCCTCCTCCTCCT10466662P4GAGCTATTCCAACGCAGTGACCACA11082263P2GTGGCTTATTGCAAGCAGCGCCTATT1044960RF 6363P1GCAACAGCGATGACCACCACA11082263P2GTGGCTTATTGCAAGCAGCTCTCGTAAC11082263P3ACCCGGATGATCATCTCCTCTCTAAC11082263P4ATCCTACGCGCCCAAGAATGTTGCAACACA11082263P3ACCCGGATGATGAATGTTGTAACGGAA11037363P4ATCCTACGCGCCCAAGATGTTGGAA110447GFPGFP-P1GAACGTCCACCGCCCCAAGAATGTTGAA110447GFPGFP-P2GATGCCTCTTCTGCTTGGAA430β-ActinActP1GATGCCATCCTCACACGCGGGGAAGATGT3339OtherLu-T7-oligo(dT)GTAAACGACGCCAGCGCCAGCACCACCACCACACACAGGAGCATGTT3309PhbactCAACGCCCACACGCGCAGCAGTATATCGACTCTATAGGGATGGTT3309PrbactCAACGCCCACACGCGCAGTGCGACGTTTTTTTTTTTTTT	ORF 29	29P1	CTACGGTTATACGTGCATTGG	54650
29P3GCCTTACAGACGACGAATTTAA5471029P4CGTTAATTACTGTAGCTCCATCGT5485029P4TGATGCCCTTTTAGAAG547500RF 4040P1GTACTTAGAACTCCGTAAC7518840P2TTGACTACAGACAGCACAAC7530140P3CCTATACAGACACGGCTACC7530140P4AGAGCTAAGTCGCACCACCACACA752290RF 6262P1CTATCATCTCCCACCGGTAG10512162P2CCACCCCCGATTAACAGCGCCTCCTC10434162P3ATGGAGTGGATGTCCCACCGCCTTAT104459Ph60GAGTGTTCCAACGGCTCTCCTCCT10436162P4GAGTGTTCCCAACGTGTCTAACCCGGT103220RF 6363P1GCACACGCACGACACA11032263P2TTGGTACAGCATCGCGCGCCTTATT104459Ph63TGACGCGCTCTCTCTCTCCTCCT10459163P2TTGGTACAGCGCCCCCCGAA11032263P3ACCCGGCTCTCTCTCACCCGCACA11032263P4ATCTACGCGCCATATGCTCTACCGAA11042764PGAGTGCTTCTCTGCCACCCCCGCAA11042764PGAGCGCTCCTCTCTGCCTCCTCTACCCACACCAC11042764PGCCCCCGCTCTCTCTGCCCCCCCCCCCCCCCCCCCCCC		29P2	GTATGAAGGTGGTGCGTGATTCTCTTCTGT	54936
29P4CGTTAATTACTGTAGCTCCATCGT54850Prb29TGATGCCGTTTTAGAAG54795ORF 4040P1GTACTTTAGAACTGCGTAAC7518840P2TTGACTTACAGACAGACAAC7522840P3CCTATACAGACAGGCTACC75529Prb40TTAAACGACCTCCTGGATCTAAC755290RF 6262P1CTATCATCTCCCACCGGTAG10434162P2CCACCCCCGATTAAACTCT10445962P3ATGGAGTGCAGTGTCTCCTCCTCTCTCT1044590RF 6363P1GCACACGCGATGAGCGACCAC11032263P4ATCTACGGCCCGGCAAGCCACCACCGGAA11032363P3ACCCGGATGAGCGACCACCGCGCAA11052363P4ATCTTAGGGCCCAAGCGACCACCGCGAA11057363P4TGCACTCATCTGCCACCGCGCAA11057363P4TGCACTCATCTGCACACCCGCGAA110447GFPGPP-P1GAACTCATCTGCTAGCACCCCGCAAA11057363P4TGCACTCATCTGCCCCCAAGAAC131 ^b GFP-P3TTCTCACTCCAGCTGTGTCCCCCAAGAA300β-ActinPrbGFPTTCGCATCACCCCCCCCAGGAAT3260 ^c β-ActinActP1GAACTCACTCTCAAGTGCGCCAGGAA3339OtherLU-17-oligo(dT)GTAAAACACACGGCCAGGTA ATACGACTCACTATAGGACTGCTT3309OtherLU-17-oligo(dt)GACACACCGCTATCGATGTCGACCCCCACGGAACTCACTATAGGACTGCTT3309OtherLU-17-oligo(dt)GACCACCGCTATCGATGTCGACCTCTTTTTTTTTTTTTT		29P3	GCCTTACAGACGACGAATATTTA	54710
Phb29TCATCGCCTTTTAGAAG54795ORF 4040P1GTACTTTAGAAG7518840P2TTGACTTACAGACAGACAAC752840P3CCTATACAGACAGGCTACC7530140P4AGACCTAACTCGCACATACA75622phb40TTAAACGACCTCCTGGATCTAAC75529ORF 6262P1CCACCCCGATTAAACTCT10434162P2CCACCCCCGATGACCTCTGCTCCTCCT10446662P4GACTGTTCCAACGTCCTTTTATTG10446662P4GACGTCTATGCAGTCGCGCGCTTATT10446662P4GACGTCTATTCCAACGTCCTCCTCCT104259765275529105221052263P3ATCGAGCGATGACGACCA11032363P4GCAACAGCGATGATCACCCGCGCTTATT1044960RF 6363P1GCAACAGCGATGATGATGCTCTCCTCCT11062263P3ACCCGGATGATGAATGTTGTACGGACA11037364PTCACGACGCCTCCCAAGATGCTCACC11052365PGFP-P1GAAGCTCATCTCGCACGCGCAA1104476FPGFP-P2GATGCCTTCTTCTGCTTGCT4826FPGFP-P3TTCTCAAGTCCGCCATGCCCAGGAT310β-ActinActP1GATGCATTGTACAGGCCCCCAGGAT3309β-ActinActP1GATGCCTCCTCAGGGGGAGATATGCACTATAGGGATCGTT3500β-ActinActP1GATGCCTCCTACGATGCGAGGAGGTG3339OtherLU-T7-oligo(dT)GTAAAACGACGCCCAGTATCGATGTCACACATATAGGGATCGTT3500β-ActinACCE-oligo(dt)GATAAACGACGCCCAGTATCGATGTCGACTCACTATAGGGATCGTT3339OtherLU-T7-oligo(dT)GTAAAACGACGCGCAGGTGATATACGACTCACATATAGGGATCGTT <td></td> <td>29P4</td> <td>CGTTAATTACTGTAGCTCCATCGT</td> <td>54850</td>		29P4	CGTTAATTACTGTAGCTCCATCGT	54850
ORF 4040P1GTACTTTAGAACTGCGTAAC7518840P2TTGACTTACAGACAGACAAC7572840P3CCTATACAGACAGGCTACC7530140P4AGAGCTAAGTCACTCGCACATACA75622Prb40TTAAACGACCTCCTGGATCTAAC75529ORF 6262P1CTATCATTCTCCACCCGCTAG10512162P2CCACCCCGATTAAACTCT10434162P3ATGGAGTGGATGCTCCGCGCTCTCT10466662P4GAGTGTTCCAACGTGCTTCCTCCTCCTC104459Prb62GTGGCTTATTGCAGTCCGGGGCTTATT10446663P363P1GCAACAGCGATGACCACCA11032263P2TTGTGTACAGACTCTCGTAACTCCGTA10459263P2TGGCTACAGACTCTCGTAACTCCGTA11032263P3ACCCGGATGATGAAGTGTTGTAACGAA11047364P4ATCTTAGCGGCCCATATGCTCTACC11059263P3ACCCGGGCCCCATATGCTCTACC110592Prb63TGACGACGGCCCCAAGCAGCAA1104776FPGAAGTCCATCTGCACACCCCCCAGAA1104776FP-P3TTCTTCAAGTCCCCCAAGCAGCAT430β-ActinActP1GAAGCCATCTTGTACGGAAGT430β-ActinActP2TCATACACATCTCAAGTTGCACACCCCCAGGAT3339OtherLU-T7-oligo(dT)GTAAAACGACGCCAAGGGGACGTG3339OtherLU-T7-oligo(dt)GACACACGCGTATGCATGCACCACTTTTTTTTTTTTTTT		Prb29	TGATGCCGTTTTAGAAG	54795
40P2TTGACTTACAGACACACACACACACACACACACACACACA	ORF 40	40P1	GTACTTTAGAACTGCGTAAC	75188
40P3CCTATACAGACAGGCTACC7530140P4AGAGCTAAGTCACTCGCACATACA75622Prb40TTAAACGACCTCCTGGATCTAAC75629ORF 6262P1CTATCATTCTCCCACCGGTAG10512162P2CCACCCCCGATTAAACTCT10434162P3GAGTGTTCCCACGTGCTCCTCCTC104459Prb62GTGGCTTATTCCAGCGGCGCTTATT104496ORF 6363P1GCAACAGCGATGACCGCGGCCTTATT1062263P3ACCCGGATGACGCACAACGTCTCCGTAACTCCGT11062263P3ACCCGGATGATGCCCGAGCACA11037363P4ATCTTAGCGCCCATATTGCTACCGAA11057363P4ATCTTAGCGCCCATAGTCGCAGCACA11057365P4TGACCACGCGTCCTCGCTACCC110447GFPGAPCCCCCTCCTCTGCCTTCTCC482GFP-P1GAAGTTCATCTGCACCCGCCAAGATGTTGAA131 ^b β-ActinGAP2TTCTTCAAGTCCGCCAAGATGTCGCAGAT3260 ^c β-ActinActP1GATGCATCTTCACAGTCGCGCGATATGCTGAGC3309OtherLU-T7-oligo(dT)GTAAAACCACGCCCAGGCAAATACCCACTATAGGGATCGTT3309OtherLU-T7-oligo(dT)GTAAAACCACGCCATGCCGACGACCACTATAGGGATCGTT3309OtherLU-T7-oligo(dT)GTAAAACCACGCCAGGTAATAACGACTCACTATAGGGATCGTT3309OtherLU-T7-oligo(dT)GTAAAACCACGCCAGGTAATGCTGCGACTTTTTTTTTTT		40P2	TTGACTTACAGACAGACAAC	75728
40P4AGAGCTAAGTCACTCGCACATACA75622Phb40TTAAACGACCTCCTGGATCTAAC75529ORF 6262P1CTATCATTCTCCCACCGCTAG10512162P2CCACCCCGATTAAACTCT10434162P3ATGGAGTGCTCCTCGTCCTCCTCCT10466662P4GAGTGTTCCAACGTGCTCTCGTCCTCT104459Phb62GTGGCTTATTGCAGCGGGGCTTATTG1042263P2TTGTGTACAGACTCTCGTAACTCCGT11052263P2TTGTGTACAGACTCTCGTAACTCCGTA11052263P3ACCCGGATGATGATGATGTTGTACGGAA11052263P4GCACAGCGCCCATATGCTCTACCCGT11052263P3ACCCGGATGATGCTCTCACACACCGGAA11057364P4ATCTTAGCGCCCATATGCTCTACCG11052265P2GAGGTCATCTTCGCTAACCCCCGCAA11057365P4GFP-P2GACGCGCGCCCCGAAGATGTTGAA110447GFPGFP-P3TTGTCACAGCTCCTCTGTGCTCGAAC24667P-P3TTGTACAAGTCCCCCAGGCAA430β-ActinGATCACATCTCAAGTTGCGCCAAGCCC450β-ActinGATCCACCCCACACGGGAAGT3200PhbGFPTTGCCCACCACACGGGAAGTGGGG3339OtherLU-T7-oligo(dT)GTAAAACGACGCCAGCTG ATATACGACTCACATATAGGGATCGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT		40P3	CCTATACAGACAGGGCTACC	75301
Phb0TTAAACGACCTCCTGGATCTAAC75529ORF 6262P1CTATCATTCTCCACACGGTAG10512162P2CCACCCCCGATTAAACTCT10436162P3ATGGAGTGGATGTCCTCGTCCTCCT10466662P4GAGTGTTCCAACGTTTTTATTG104496ORF 6363P1GCAACAGCGATGACGACCA11032263P2TTGTGTACAGACTCTCGTAACTCCGT11062263P3ACCCGGATGAACGATGTTGTAACGAAA11037363P4ATCTTAGCGGCCAATGTTGAAC110542Phb63TGACGACGGCTCCGAAGATGTTGAA110447GFPGFP-P1GAACGACGCTCCGAACATGTTGAA1105426FP-P2GATCCCGTCTTGCTTGCTTGCAA1105426FP-P3TTGTCAACGGCTCCCCAAGATGTTGAA110447GFPGFP-P4TGACGACGCTCCCGAAGATGTTGAA104476FP-P3TGCCGTCTCTTGCTTGCTTGCT4826FP-P4TTGTCACACCTGCCCATGGCC430β-ActinActP1GATGCATCGTCACGAGGTGCCAAGATGTGAGATG3500PhbctCAAGTCCACACAGGCGAGGTG3500PhbActCAAGTCCACACAGGCGAGGTG3509PhbActGACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTTTT		40P4	AGAGCTAAGTCACTCGCACATACA	75622
ORF 6262P1CTATCATTCTCCCACCGGTAG10512162P2CCACCCCGATTAAACTCT10434162P3ATGGAGTGGATGTCCTCGTCCTCCT10466662P4GAGTGTTCCAACGTCTTTTATTG104459Prb62GTGGCTTATTGCAGCGCGGGCTTATT10426ORF 6363P1GCAACAGCGATGACGACCA11032263P2TTGTGTACAGAGCTCTGTAACTCCGT11062263P3ACCCGGATGATGAATGTTGTACGGAA11037363P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGGCTCCGAACATGTTGAA110447GFPGAAGTTCATCTGCACCACCGCGCAA110447GFPGATGCCGTTCTTCTGCTTGTC482GFP-P3TTCTTCAAGTCCGCCATGCCC246GFP-P4TTGTACTCCAGCTTGTCCCCAGGAT430Prb6FPTTGCCGTCCTTGTGCCCCAGGAT300β-ActinActP1GATGCCATTGTTACAGGAAGT3260°ActP2TCATACATCTCAAGTTGGGGG3500PrbActCAAGTCCACACAGGGCAGGTG3339OtherLu-T7-oligo(dT)GTAAAACGACGGCAGGTGCACCATTTTTTTTTTTTTTTT		Prb40	TTAAACGACCTCCTGGATCTAAC	75529
62P2CCACCCCGATTAAACTCT10434162P3ATGGAGTGGATGCTCCTCGTCCTCCT10466662P4GAGTGTTCCAACGTGTTTAATTG104459Prb62GTGGCTTATTGCAGTGCGGGGCTTATT104296ORF 6363P1GCAACAGCGATGACGACCA11032263P2TTGTGTACAGAGTCTCGTAACTCCGT11062263P3ACCCGGATGATGACAATGTTGTACGGAA11037363P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGGCTCCGAAGATGTTGAA110447GFPGFP-P1GAAGTCACTCGCTTGCTCGCCCAGGAA110447GFPGFP-P2GATGCCGTTCTTCTGCACCACCGGCAA131 ^b GFP-P3TTCTTCAAGTCCGCCATGCCC482GFP-P4TTGTACTCCAGCTTGTGCCCCAGGAT430PrbGFFTTGCCGTCCTCCTTGAAGTCGATG403β-ActinGATGCATTGTACAGGAGAGT3500prbActCAAGTCCAACACAGGGAGAGTG3339OtherLu-Tr-oligo(dT)GTAAAACGACGGCAGGTAATACGACTCAATAGGATCGTTGTTTTTTTT	ORF 62	62P1	CTATCATTCTCCCACCGGTAG	105121
62P3ATGGAGTGGATGTCCTCGTCCTCCT10466662P4GAGTGTTCCAACGTGTTTTATTG104459Prb62GTGGCTTATTGCAGTCGCGGGCTTATT104496ORF 6363P1GCAACAGCGATGACGACCA11032263P2TTGTGTACAGACTCTCGTAACTCCGT11062263P3ACCCGGATGATGAATGTTCTACGGAA11037363P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGCCTCCGAACATGTTGAA110447GFPGFP-P1GAAGTCACTCGCGCCAAGATGTTGAA110477GFP-P2GATGCCGTTCTTCTGCTTGTC482GFP-P3TTCTTCAAGTCCGCCCATGCCC246GFP-P4TGGCGTCCTCTTGTGTGTC430β-ActinActP1GATGCCATTGTACAGGATGTGGAGTGGATG3309β-ActinActP1GATGCCATCTCAAGTGGGGG3309OtherLu-Tr-oligo(dT)GATAACAGCGCGATGGCGAATATAGCACTCATATAGGGATGTT3339OtherRACE-oligo(dt)GACCACGCGTATCGATGTGAACTACACATATGGATGTT339ACEGACACAGCGCTATCGATGCGACGACGACGACGACGACGACGACGACGACGACGACGA		62P2	CCACCCCGATTAAACTCT	104341
62P4GAGTGTTCCAACGTGTTTATTG104459Prb62GTGGCTTATTGCAGTCGCGGGGCTTATT104496ORF 6363P1GCAACAGCGATGACGACCA11032263P2TTGTGTACAGACTCTCGTAACTCCGT11062263P3ACCCGGATGATGATATGTTGTACGGAA11037363P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGGCTCCGAAGATGTTGAA131 ^b GFP-1GAAGTTCATCTGCACCACCGGCAA131 ^b GFP-P2GATGCCGTCTTCTGTGTTGTC482GFP-P3TTCTTCAAGTCCGCCATGCCC266GFP-P4TTGTACTCCAGCTTGTGCCCAGGAT430β-ActinActP1GATGCATTGTACAGGAAGT3260°ActP2TCATACATCTCAAGTCGGGG3500PrbActCAAGTCCACACAGGGGAGGTG3339OtherU-T7-oligo(dT)GTAAAACGACGCCATGGCGCATATAGGATCGTT3339ACE-oligo(dt)GACCACGCGTATCGATGTCGACGTTTTTTTTTTTTTTTT		62P3	ATGGAGTGGATGTCCTCGTCCTCCT	104666
Prb62GTGGCTTATTGCAGTCGCGGGCTTATT104496ORF 6363P1GCAACAGCGATGACGACCA11032263P2TTGTGTACAGACTCTCGTAACTCCGT11062263P3ACCCGGATGATGAATGTTGTACGGAA11037363P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGGCTCCGAAGATGTTGAA110447GFPGFP-P1GAAGTTCATCTGCACCACCGGCAA131 ^b GFP-P2GATGCCGTTCTTCTGCTTGTC482GFP-P3TTGTAAGCGCCATGCCCCAGGAT430β-ActinActP1GATGCATTGTTACAGGAAGT3260 ^c ActP2TCATACATCTCAAGTTGGGGG3339OtherLU-77-oligo(dT)GACCACGCGTATCGATGTCGACGACTCATATAGGGATCGTT3339OtherRACE-oligo(dt)GACCACGCGTATCGATGTCGACGACT3339		62P4	GAGTGTTCCAACGTGTTTTATTG	104459
ORF 6363P1GCAACAGCGATGACGACCA11032263P2TTGTGTACAGACTCTCGTAACTCCGT11062263P3ACCCGGATGATGATGAATGTTGTACGGAA11037363P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGGCTCCGAAGATGTTGAA110447GFPGFP-P1GAAGTTCATCTGCACCACCGGCAA131 ^b GFP-P2GATGCCGTTCTTCTGCTTGTC482GFP-P3TTCTTCAAGTCCGCCATGCCC246GFP-P4TTGTACTCCAGCTTGTGCCCCAGGAT430PrbGFPTTGCCGTCCTCCTTGAAGTCGATGT3260°β-ActinActP1GATGCATTGTTACAGGAAGT3260°ActP2TCATACATCTCAAGTTCGGGGG3339OtherLU-T7-oligo(dT)GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT3339OtherKACE-oligo(dt)GACCACGCGTATCGATGTCGACC3339ACEGACCACGCGTATCGATGTCGACCGACCACGCGTATCGATGTCGAC339		Prb62	GTGGCTTATTGCAGTCGCGGGCTTATT	104496
63P2TTGTGTACAGACTCTCGTAACTCCGT11062263P3ACCCGGATGATGAATGTTGTACGGAA11037363P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGGCTCCGAAGATGTTGAA110447GFPGAFP-P1GAAGTTCATCTGCACCACCGGCAA131 ^b GFP-P2GATGCCGTTCTTCTGCTTGTC482GFP-P3TTCTTCAAGTCCGCCATGCCC246FP-P4TTGTACTCCAGCTTGTGCCCCAGGAT430β-ActinActP1GATGCATTGTTACAGGAAGT3260 ^c ActP2TCATACATCTCAAGTTGGGGG3500PrbActCAAGTCCACACAGGGAGGTG3339OtherLU-T7-oligo(dT)GACCACGCGTATCGATGTCGACGACCATATAGGGATCGTT3339ACEGACCACGCGTATCGATGTCGACGTCGACG3349	ORF 63	63P1	GCAACAGCGATGACGACCA	110322
63P3ACCCGGATGATGATGTTGTACGGAA11037363P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGGCTCCGAAGATGTTGAA110447GFPGFP-P1GAAGTTCATCTGCACCACCGGCAA131 ^b GFP-P2GATGCCGTTCTTCTGCTTGTC482GFP-P3TTCTCAAGTCCGCCATGCCC246GFP-P4TTGTACTCCAGCTTGTGCCCAGGAT403β-ActinActP1GATGCCATTGTTACAGGTCGATG3260 ^c ActP2TCATACATCTCAAGTTGGGGG3500PrbActCAAGTCCACAGGGCAGGTG3339OtherLU-T7-oligo(dT)GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT		63P2	TTGTGTACAGACTCTCGTAACTCCGT	110622
63P4ATCTTAGCGGCCATATGCTCTACC110592Prb63TGACGACGGCTCCGAAGATGTTGAA110447GFPGFP-P1GAAGTTCATCTGCACCACCGGCAA131 ^b GFP-P2GATGCCGTTCTTCTGCTTGTC482GFP-P3TTCTTCAAGTCCGCCATGCCC246GFP-P4TTGTACTCCAGCTTGTGCCCAGGAT430β-ActinActP1GATGCCATTGTTACAGGAAGAT3260°ActP2TCATACATCTCAAGTCGGCG3500PrbActCAAGTCCACACGGGCAGGTG3339OtherLU-T7-oligo(dT)GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT		63P3	ACCCGGATGATGAATGTTGTACGGAA	110373
Prb63TGACGACGGCTCCGAAGATGTTGAA110447GFPGFP-P1GAAGTTCATCTGCACCACCGGCAA131 ^b GFP-P2GATGCCGTTCTTCTGCTTGTC482GFP-P3TTCTTCAAGTCCGCCATGCCC246GFP-P4TTGTACTCCAGCTTGTGCCCCAGGAT430PrbGFPTTGCCGTCCTCTTGAAGTCGAAGTG403β-ActinActP1GATGCATTGTTACAGGAAGT3260 ^c ActP2CATACATCTCAAGTTGGGGG3500PrbActCAAGTCCAACAGGGGAAGTG339OtherLU-T7-oligo(dT)GTAAAACGACGGCCAGGTAATAACGACTCACTATAGGGATCGTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT		63P4	ATCTTAGCGGCCATATGCTCTACC	110592
$ \begin{array}{llllllllllllllllllllllllllllllllllll$		Prb63	TGACGACGGCTCCGAAGATGTTGAA	110447
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	GFP	GFP-P1	GAAGTTCATCTGCACCACCGGCAA	131^{b}
$ \begin{array}{cccc} {\rm GFP-P3} & {\rm TTCTTCAAGTCCGCCATGCCC} & 246 \\ {\rm GFP-P4} & {\rm TTGTACTCCAGCTTGTGCCCCAGGAT} & 430 \\ {\rm PrbGFP} & {\rm TTGCAGTCTCTTGAAGTCGATG} & 403 \\ {\rm \rhorbGFP} & {\rm TTGCCGTCTCTTGAAGTCGATG} & 3260^{\circ} \\ {\rm ActP1} & {\rm GATGCATGTTACAGGAAGT} & 3260^{\circ} \\ {\rm ActP2} & {\rm TCATACATCTCAAGTTGGGGG} & 3500 \\ {\rm PrbAct} & {\rm CAAGTCCAACACGGGAGGTG} & 3339 \\ \\ {\rm Other} & {\rm LU-T7-oligo(dT)} & {\rm GACACGCGTATCGATGTCGACGACTCACTATAGGGATCGTT} \\ {\rm RACE-oligo(dt)} & {\rm GACCACGCGTATCGATGTCGAC} \\ {\rm RACE} & {\rm GACCACGCGTATCGATGTCGAC} \\ \end{array} $		GFP-P2	GATGCCGTTCTTCTGCTTGTC	482
$ \begin{array}{cccc} {\rm GFP-P4} & {\rm TTGTACTCCAGCTTGTGCCCCAGGAT} & 430 \\ {\rm PrbGFP} & {\rm TTGCCGTCCTCGTGAAGTCGATG} & 403 \\ {\rm ActP1} & {\rm GATGCATGTTACAGGAAGT} & 3260^{\rm c} \\ {\rm ActP2} & {\rm TCATACATCTCAAGTTGGGGG} & 3500 \\ {\rm PrbAct} & {\rm CAAGTCCAACATGGGGGGG} & 3339 \\ {\rm Other} & {\rm LU-T7\-oligo(dT)} & {\rm GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT} \\ {\rm RACE\-oligo(dt)} & {\rm GACCACGCGTATCGATGTCGAC} & {\rm STTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT$		GFP-P3	TTCTTCAAGTCCGCCATGCCC	246
$ \begin{array}{cccc} & \mbox{PrbGFP} & \mbox{TTGCCGTCCTTGAAGTCGATG} & \mbox{403} \\ & \mbox{ActP1} & \mbox{GATGCATTGTTACAGGAAGT} & \mbox{3260}^c \\ & \mbox{ActP2} & \mbox{TCATACATCTCAAGTTGGGGG} & \mbox{3500} \\ & \mbox{PrbAct} & \mbox{CAAGTCCACAGGGGAGGTG} & \mbox{3339} \\ \\ \mbox{Other} & \mbox{LU-T7-oligo(dT)} & \mbox{GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT} \\ & \mbox{RACE-oligo(dt)} & \mbox{GACCACGGGTATCGATGTCGAC} & FTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT$		GFP-P4	TTGTACTCCAGCTTGTGCCCCAGGAT	430
$ \begin{array}{cccc} \beta - Actin & ActP1 & GATGCATTGTTACAGGAAGT & 3260^c \\ ActP2 & TCATACATCTCAAGTTGGGGG & 3500 \\ PrbAct & CAAGTCCACAGGGGAGGTG & 3339 \\ Other & LU-T7-oligo(dT) & GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT \\ TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT$		PrbGFP	TTGCCGTCCTTCGAAGTCGATG	403
ActP2TCATACATCTCAAGTTGGGGG3500PrbActCAAGTCCACAGGGGAGGTG3339OtherLU-T7-oligo(dT)GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT TTTTTTTTTTTTTTTTTTTTTRACE-oligo(dt)GACCACGCGTATCGATGTCGACRACEGACCACGCGTATCGATGTCGAC	β -Actin	ActP1	GATGCATTGTTACAGGAAGT	3260°
PrbAct CAAGTCCACAGGGGAGGTG 3339 Other LU-T7-oligo(dT) GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT TTTTTTTTTT		ActP2	TCATACATCTCAAGTTGGGGG	3500
Other LU-T7-oligo(dT) GTAAAACGACGGCCAGGTAATACGACTCACTATAGGGATCGTT TTTTTTTTTT		PrbAct	CAAGTCCACAGGGGGAGGTG	3339
RACE-oligo(dt)GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTTTT	Other	LU-T7-oligo(dT)	GTAAAACGACGGCCAGGTA ATACGACTCACTATAGGGATCGTT TTTTTTTTTTTTT	
RACE GACCACGCGTATCGATGTCGAC		RACE-oligo(dt)	GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTTTT	
		RACE	GACCACGCGTATCGATGTCGAC	

^aLocation of the 5′ nucleotide on the SVV genome (GenBank Accession No. AF275348) (Gray *et al*, 1995).

^bLocation of the 5' nucleotide with respect to the GFP DNA sequence supplied by Clontech.

^cLocation of the 5' nucleotide with respect to the β -actin cDNA sequence (Nakajima-Iijima *et al*, 1985).

 ${}^{d}N = nucleotide A, G, or C.$

DNA electrophoresis, Southern blotting, and DNA hybridization

PCR and RT-PCR DNA amplification products were resolved by electrophoresis through a 2% agarose gel, electrophoretically transferred to a Zeta-probe membrane (BIO-RAD, Laboratories, Hercules, CA) and hybridized overnight at 42°C to the appropriate ³²P-end-labeled internal oligonucleotide (Table 3). Oligonucleotides were end-labeled with [³²P]dATP using T4 kinase (Life Technologies, Inc) according to the manufacturer's instructions. Probed Southern blots were processed as described (LaGuardia *et al*, 1999). Images were developed by exposure to a PhosphorImager and analyzed using ImageQuant software (Molecular Dynamics, Inc, Sunnyvale, CA).

ORF	Primary	Nested	Probe	Product length
SVV 4	4P1/4P2	4P3/4P4	Prb4	232 bp
SVV 21	21P1/21P2	21P3/21P4	Prb21A	111 bp
	21P5/21P6	21P7/21P8	Prb21B	172 bp
SVV 28	28P1/28P2	28P3/28P4	Prb28	$230\mathrm{bp}$
SVV 29	29P1/28P2	29P3/29P4	Prb29	$140 \mathrm{bp}$
SVV 40	40P1/40P2	40P3/40P4	Prb40	323 bp
SVV 62	62P1/62P2	62P3/62P4	Prb62	207 bp
SVV 63	63P1/63P2	63P3/63P4	Prb63	219 bp
Actin	ActP1/ActP2	_	PrbAct	240 bp
(DNA)				-
Actin	ActP1/RACE	_	PrbAct	369 bp
(cDNA)				-
GFP	GFP-P1/GFP-P2	GFP-P3/GFP-P4	PrbGFP	184 bp

Preparation of preimmune rabbit IgG and rabbit anti-SVV IgG

Rabbit anti-SVV antiserum was prepared by subcutaneous inoculation of purified SV virions as described for rabbit anti-VZV antiserum (Wroblewska et al, 1982). IgG was prepared from preimmune rabbit serum and rabbit anti-SVV antiserum. One ml of preimmune and hyperimmune sample was passed through a protein A-bead column twice. Beads were washed with 10 column volumes of 100 mM Tris (pH 8.0) and 10 column volumes of 10 mM Tris (pH 8.0) followed by one wash with 0.5 ml ddH_2O . IgG was eluted stepwise with 500 μ l of 100 mM glycine (pH 3.0). Eluates were collected in 1.5-ml Eppendorf tubes containing 50 μ l of 1 M Tris (pH 8.0). IgG-containing fractions were identified by absorbance at 280 nm and pooled. Concentration of the pooled fractions was determined using the BCA protein assay (Pierce, Rockford, IL).

Immunohistochemistry

Sections (6–8 μ m) of frozen monkey ganglia and liver were fixed in cold acetone and stained with hematoxylin and eosin (H&E). Other sections were analyzed by immunohistochemistry with preimmune rabbit IgG, rabbit anti-SVV IgG, or a mouse antineurofilament monoclonal antibody (mAb) (clone N52; SIGMA, St. Louis, MO). The preimmune rabbit IgG and rabbit anti-SVV IgG were preabsorbed twice on BSC-1 cells and twice on acetone-dried uninfected monkey ganglia powder for 1 h at 37°C followed by 20 h at 4°C with mixing. Unless indicated, all incubations were at room temperature. Fixed sections were incubated with a 10% (vol/vol) solution of normal goat serum for 1 h, washed three times with phosphate-buffered saline (PBS), and incubated for 1 h with preimmune rabbit IgG (2 μ g/ml), rabbit anti-SVV IgG (2 μ g/ml) or a 1:2500 dilution of mouse anti-neurofilament mAb in PBS. Sections were washed three times with PBS, incubated for 1 h with a 1:500 dilution of goat anti-rabbit IgG (Vector Laboratories, Inc, Burlingame, CA) or a 1:400 dilution

of alkaline phosphatase-conjugated horse anti-mouse IgG in PBS containing 5% normal goat serum and washed three times with PBS. The color reaction was developed for 5–10 min with fresh fuchsin substrate system (code K698; DAKO, Carpinteria, CA). One drop of 125 mM levamisole (Vector Laboratories, Inc) was added to the reaction mix to block endogenous phosphatases. Acetone-fixed SVV-infected and uninfected BSC-1 cells on coverslips were treated identically.

Results

Sensitivity of nested PCR used to detect SVV DNA DNA extracted from liver of an SVV-seronegative monkey was spiked with 0, 1, 10, 100, or 1000 copies of the previously described cloned SVV restriction fragments, EcoRI G, Pst N, and EcoRI C (Clarke et al, 1992) to determine the sensitivity of the primer sets used in this study. SVV EcoRI G, Pst N, and EcoRI C contain SVV ORF 4-, 21-, and 63-specific sequences respectively. Liver DNA (0.2 μ g) from an uninfected monkey was mixed with 0, 1, 10, 100, or 1000 copies of the prior SVV restriction fragments and analyzed by nested PCR using SVV ORF 4-, 21-, or 63-specific primers followed by Southern blot hybridization of nested PCR amplification products (Figure 2). The lower limit of detection was 1 copy using primer sets specific for SVV ORFs 4 or 63, and 10 copies using a primer set specific for SVV ORF 21.

Sensitivity of the nested RT-PCR procedure used to detect SVV-specific transcripts

Total RNA extracted from ganglia was spiked with 1, 10, 100, 1000, or 10000 copies of *in vitro* synthesized transcripts specific for green fluorescent protein (GFP) to determine the sensitivity of the nested RT-PCR procedure used in this study. The gene that



Figure 2 Sensitivity of nested PCR used to detect multiple regions of the SVV genome. Southern blot showing detection of 0, 1, 10, 100, and 1000 copies of SVV DNA sequences representing ORFs 4, 21, and 63. One, 10, 100, or 1000 copies of plasmid DNA containing each SVV-specific sequence was mixed with 0.2 μ g of DNA extracted from the liver of an SVV-seronegative African green monkey. The DNA mixture was analyzed by nested PCR followed by Southern blot hybridization of the amplification products using internal SVV-specific oligonucleotides as described in Materials and methods.



Figure 3 Sensitivity of nested RT-PCR. Southern blot showing detection of 0, 1, 10, 100, 1000, and 10000 copies of *in vitro* synthesized transcripts specific for green fluorescent protein (GFP). Sensitivity of the nested RT-PCR reaction was determined using ganglionic RNA of an African green monkey spiked with known copies *in vitro* GFP transcripts prior to RT reaction. Each RNA sample was analyzed by nested RT-PCR followed by Southern blot hybridization of the amplification products using an internal GFP-specific oligonucleotide as described in Materials and methods.

encodes GFP was used because of its lack of homology to African green monkey genomic DNA. Therefore, there was no concern that the GFP-specific primers would amplify cellular cDNA sequences. The RNA mixture was analyzed by RT-PCR followed by Southern blot hybridization of the nested amplification product (Figure 3). The RT-PCR detected 10 to 10000 copies of *in vitro* GFP transcripts.

Detection of SVV DNA sequences in ganglia, lung, and liver

SVV DNA sequences representing at least one of the three ORFs (4, 21, or 63) were detected in ganglia from all monkeys (M1–M12) 10 and 11 days p.i., and 2, 5, 10, and 12 months p.i. (Table 4). DNA sequences representing at least one of the three SVV ORFs (4, 21, or 63) were detected in lung at 10 and 11 days p.i. and 2, 5, and 10 months p.i. (M1–M4, M6–M11; Table 4). SVV DNA sequences were not detected in lung of monkeys M5 and M12 (2 and 12 months p.i.). DNA sequences representing at least one of the three SVV ORFs (4, 21, or 63) were detected in liver of monkeys M1–M10 (10 and 11 days p.i., and 2, 5, and 10 months p.i.); livers from monkeys M11 and M12

were negative for SVV ORFs 4 and 21, respectively (Table 4). SVV ORF 4, 21, and 63 DNA sequences were not detected in ganglia, lung, and liver from an SVVseronegative adult African green monkey (Table 4). Actin sequences were detected in all DNA.

Detection of SVV-specific transcripts in ganglia, lung. and liver

SVV IE ORF 4-, 62-, 63-, E ORF 21-, 28-, 29-, or L ORF 40-specific transcripts were detected in ganglia, lung, and liver of 11 SVV-infected monkeys (M2-M12) at 11 days, 2, 5, 10, and 12 months p.i. (Tables 5-8). SVV-IE ORF 4-, 62-, 63-, E ORF 21-, 28-, 29-, and L ORF 40-specific were not detected in RNA extracted from ganglia, lung, or liver of an SVV-seronegative monkey (Figure 4). We analyzed the RNA extracted from the monkey tissues using RT followed by nested PCR and Southern blot hybridization. The strong intensity of the bands obtained after hybridization of the nested RT PCR product indicated that the detection of SVV transcripts was not due to a low level of false starts that could have produced partial RNAs. Actin-specific transcripts were detected in all RNA samples from liver and lung.

 $\label{eq:table_$

	Sacrificed (time p.i.)		Ganglia			Lung		Liver		
Monkey		4	21	63	4	21	63	4	21	63
SVV-seronegative		_	_	_	_	_	_	_	_	_
M1	10 days	nd^a	nd	+	nd	nd	+	nd	nd	+
M2	11 days	+	+	+	+	+	+	+	+	+
M3	2 mo	nd	+	nd	nd	+	nd	nd	+	nd
M4	2 mo	+	+	+	+	+	+	_	+	+
M5	2 mo	nd	+	nd	nd	_	nd	nd	+	nd
M6	5 mo	+	+	+	+	+	+	_	+	_
M7	5 mo	+	+	nd	_	+	nd	+	+	nd
M8	5 mo	+	+	+	+	+	+	_	+	_
M9	10 mo	+	+	+	+	+	+	_	+	_
M10	10 mo	+	+	nd	_	+	_	+	+	nd
M11	10 mo	+	+	nd	_	nd	+	_	nd	nd
M12	12 mo	nd	+	+	nd	_	nd	nd	_	nd

and = not done.

Table 5	Detection of SVV	ORF- and	actin-specific	transcripts in a	nultipl	e tissues	by nested	I RT-PCR	after int	ratrachea	l inocul	lation of
adult Af	rican green monkey	vs with 10^3	–10⁴ PFU of S	VV	1		5					

		Gai	nglia		Lu	ng		Liver				
ORF	<i>M3</i> ^a	$M4^{ m a}$	$M5^{ m a}$	$M2^{ m b}$	<i>M3</i>	M4	M5	M2	<i>M3</i>	M4	M5	M2
IE 4	+	+	+	+	\mathbf{nd}^{c}	nd	nd	+	nd	nd	nd	+
IE 62	+	+	+	+	nd	nd	nd	+	nd	nd	nd	+
IE 63	+	+	+	+	_	_	_	+	nd	_	+	+
E 21	_	+	+	+	nd	nd	nd	+	nd	nd	nd	+
E 28	_	+	+	+	_	_	_	+	nd	_	+	+
E 29	+	+	+	+	_	_	_	+	nd	_	+	+
L 40	_	_	_	+	_	_	_	+	nd	_	+	+
Actin	+	+	+	+	+	+	+	+	nd	+	+	+

^a2 months, ^b11 days p.i.

 c nd = not done.

ORF		Gai	nglia			Lu	ng	Liver				
	<i>M6</i> ^a	$M7^{ m a}$	<i>M8</i> ª	$M2^{\mathrm{b}}$	<i>M6</i>	<i>M7</i>	<i>M8</i>	M2	M6	<i>M</i> 7	<i>M8</i>	M2
IE 4	+	+	+	+	nd^{c}	nd	nd	+	nd	nd	nd	+
IE 62	+	+	+	+	nd	nd	nd	+	nd	nd	nd	+
IE 63	+	+	+	+	_	_	_	+	_	+	_	+
E 21	+	+	+	+	nd	nd	nd	+	nd	nd	nd	+
E 28	+	+	+	+	_	_	_	+	_	+	_	+
E 29	+	+	+	+	_	_	_	+	_	+	_	+
L 40	+	+	+	+	_	_	_	+	_	+	_	+
Actin	+	+	+	+	+	+	+	+	nd	+	nd	+

a5 months, b11 days p.i. cnd = not done.

Table 7 Detection of SVV ORF- and actin-specific transcripts in multiple tissues by nested RT-PCR after intratracheal inoculation of adult African green monkeys with 10³-10⁴ pfu of SVV

Ganglia						Lung						Liver				
ORF	$M9^{\mathrm{a}}$	$M10^{\mathrm{a}}$	<i>M11</i> ^a	$M12^{\mathrm{b}}$	<i>M2</i> °	M9	M10	M11	M12	M2	M9	M10	M11	M12	M2	
IE 4	+	+	+	+	+	+	\mathbf{nd}^{d}	+	_	+	nd	_	nd	_	+	
IE 62	+	+	_	+	+	_	nd	_	nd	+	nd	_	nd	nd	+	
IE 63	+	+	+	+	+	+	nd	+	nd	+	nd	+	nd	nd	+	
E 21	+	+	+	+	+	+	nd	+	nd	+	nd	+	nd	nd	+	
E 28	+	+	+	+	+	_	nd	_	_	+	nd	_	nd	_	+	
E 29	+	+	+	+	+	+	nd	+	_	+	nd	+	nd	_	+	
L 40	+	+	+	+	+	_	nd	_	_	+	nd	_	nd	_	+	
Actin	+	+	+	+	+	+	nd	+	+	+	nd	+	nd	+	+	

^a10 months, ^b12 months, ^c11 days p.i.

 d nd = not done.

Table 8 Detection of SVV ORF-specific transcripts in ganglia, lung, or liver in SVV-infected adult African green monkeys 2, 5, 10, and 12 months p.i.

		Gan	glia			Lu	ng		Liver			
ORF	2	5	10	12	2	5	10	12	2	5	10	12
IE 4	3/3ª	3/3	3/3	1/1	nd^{b}	nd	2/2	0/1	nd	nd	0/1	0/1
IE 62	3/3	3/3	2/3	1/1	nd	nd	0/2	nd	nd	nd	0/1	nd
IE 63	3/3	3/3	3/3	1/1	0/3	0/3	2/2	nd	1/2	1/3	1/1	nd
E 21	2/3	3/3	3/3	1/1	nd	nd	2/2	nd	nd	nd	1/1	nd
E 28	2/3	3/3	3/3	1/1	0/3	0/3	0/2	0/1	1/2	1/3	0/1	0/1
E 29	3/3	3/3	3/3	1/1	0/3	0/3	2/2	0/1	1/2	1/3	1/1	0/1
L 40	0/3	3/3	3/3	1/1	0/3	0/3	0/2	0/1	1/2	1/3	0/1	0/1

^aNumber of animals positive/number analyzed.

 b nd = not done.



Figure 4 Detection of actin and SVV ORF 4-, 21-, 28-, 29-, 40-, 62-, and 63-specific transcripts in multiple monkey tissues of adult African green monkeys inoculated intratracheally with 10³ pfu of SVV and sacrificed at 11 days or 10 months p.i. No SVV-specific transcripts were detected in multiple tissues from an adult SVV-seronegative African green monkey. Total RNA extracted from each tissue was analyzed by RT-PCR followed by Southern blot hybridization of nested PCR amplification products using internal actin and SVV-specific oligonucleotides as described in Materials and methods.

Actin RT-PCR was not performed on the RNA from ganglia since most of them were positive for SVV-specific transcripts which indicated the presence of amplifiable RNA. No actin- or SVV-specific amplification products were detected in reactions containing RNA that had not been reverse-transcribed.

Eleven days p.i. SVV IE ORF 4-, 62-, 63-, E ORF 21-, 28-, 29-, and L ORF 40-specific transcripts were detected in ganglia, lung, and liver of monkey M2 (Figure 4; Tables 5–8).

Two months p.i. In monkeys M3–M5, SVV IE ORF 4-, 62-, 63-, and E ORF 29-specific transcripts were detected in ganglia from all three animals. SVV transcripts representing E ORFs 21 and 28 were detected in ganglia from two of three animals. No L ORF 40-specific transcripts were detected in any ganglia, and no IE, E, or L gene transcripts were detected in lung from any of the 3 monkeys. SVV IE ORF 63-, E ORF 28-, 29-, and L ORF 40-specific transcripts were detected in the liver of monkey M5 (Tables 5 and 8).

Five months p.i. In monkeys M6–M8, SVV IE ORF 4-, 62-, 63-, E ORF 21-, 28-, 29-, and L ORF 40-specific transcripts were detected in ganglia from all three monkeys. No IE, E, or L gene transcripts were

detected in RNA from lung of any monkey, or in RNA from liver of monkeys M6 and M8. SVV IE ORF 63-, E ORF 28-, 29-, and L ORF 40-specific transcripts were detected in the liver of monkey M7 (Tables 6 and 8).

Ten months p.i. In monkeys M9–M11, SVV IE ORF 4-, 63-, E ORF 21-, 28-, 29-, and L ORF 40-specific transcripts were detected in ganglia from all three monkeys. IE ORF 62 transcripts were detected in ganglia of monkeys M9 and M10, but not in ganglia of M11. In the lung of monkeys M9 and M11, SVV transcripts representing IE ORFs 4 and 63 and E ORFs 21 and 29, but not SVV IE ORF 62-, E ORF 28- or L ORF 40-specific transcripts, were detected. SVV IE ORF 63- and E ORF 21- and 29-specific transcripts were detected in liver of monkey M10, but not SVV IE ORF 4-, 62-, E ORF 28-, or L ORF 40-specific transcripts (Figure 4; Tables 7 and 8). RNAs from the lung of monkey M10 and livers of monkeys M9 and M11 were not analyzed.

Twelve months p.i. In monkey M12, SVV IE ORF 4-, 62-, 63-, E ORF 21-, 28-, 29-, and L ORF 40-specific transcripts were detected in ganglia. No SVV IE ORF 4-, E ORF 28- or 29-, or L ORF 40-specific transcripts were detected in lung or liver (Tables 7 and 8).

Although we did not analyze every tissue from every monkey with all SVV ORFs, the data indicate that intratracheal inoculation of SVV in African green monkeys results in the presence of viral DNA and transcription of multiple viral genes in many tissues for months after experimental infection.

Immunohistochemistry and immunocytochemistry

Serial sections (6–8 μ m) of frozen liver from an SVVseronegative monkey and monkey M1 (10 days p.i.) were examined for viral antigens. SVV-specific antigens were detected throughout liver from monkey M1 (Figure 5A), in SVV-infected cells in tissue culture, but not in liver from an uninfected monkey (Figure 5B) or in uninfected cells in culture (data not shown). No staining was observed on adjacent sections of each liver sample, uninfected BSC-1, or SVV-infected cells using control preimmune rabbit IgG (data not shown).

Serial frozen sections of ganglia from two SVVseronegative monkeys and SVV-infected monkeys M1 (10 days p.i.) and M12 (12 months p.i.) were stained with H&E and analyzed by immunohistochemistry using anti-SVV IgG. No inflammation or inclusions were seen in the ganglia of monkey M1 (Figure 6A), in ganglia, lung, and liver from two SVVseronegative monkeys, or in ganglia from monkey M12 (data not shown). SVV-specific antigens were detected by immunohistochemistry in six different sections of a dorsal root ganglion from monkey M1 (Figure 6B), but not in one section of a trigeminal ganglion from an SVV-seronegative monkey (data not shown), or in two sections of a ganglion of monkey M12, even though adjacent sections from the same ganglion of M12 were positive for SVV DNA by PCR (data not shown). No staining was observed on adjacent



Figure 5 Immunohistochemical analysis of liver from SVVinfected (M1) and uninfected monkey, 10 days p.i. Indirect alkaline phosphatase staining using a 1:5000 dilution of rabbit anti-SVV IgG. (A) Note numerous dark-staining cells containing SVV-specific antigen in SVV-infected liver. (B) No SVV-specific antigen was observed in liver from an uninfected monkey. $216 \times .$

ganglionic sections of each sample using control preimmune rabbit IgG.

Staining of adjacent ganglia sections with an antineurofilament mAb revealed neurofilament protein in the cytoplasm of ganglionic cells of all monkeys tested: M1 (10 days p.i.) (Figure 6C), M12 (12 months p.i.), and an SVV-seronegative monkey (data not shown).

Overall, SVV DNA sequences were detected in all ganglia up to 12 months p.i. and in most lungs and livers for 10 months p.i. (Table 4). SVV-specific transcripts representing all gene classes were detected in ganglia 5, 10, and 12 months p.i. SVV-specific IE, E, and L transcripts were also detected 2 and 5 months p.i. in liver, and SVV-specific IE and E transcripts in liver and lung 10 months p.i. SVV-specific antigens were detected in liver and ganglia 10 days p.i., but not in ganglia 12 months p.i.

Discussion

Herein, we analyzed the molecular events after resolution of acute SVV infection in monkeys produced by intratracheal inoculation. A particular advantage

Simian varicella virus persists after experimental infection $\underline{\mathsf{TM}}$ White $\mathit{et al}$



Figure 6 Dorsal root ganglion of SVV-infected monkey M1 (10 days p.i.). (A) Hematoxylin and eosin-stained section showing a large neuron (closed arrow) and smaller non-neuronal cells (open arrow). No inflammation was seen. (B) Indirect alkaline phosphatase staining with 1:5000 dilution of rabbit anti-SVV IgG. Note dark SVV-specific antigen staining in area around a neuron (closed arrow). (C) Indirect alkaline phosphatase staining on an adjacent ganglia section with 1:2500 dilution of mouse anti-neurofilament mAb. Note dark staining of neurofilament protein in cytoplasm of ganglionic cells (closed arrow). 216×.

of our model system is that SVV-infected tissues were harvested immediately after death, thus obviating concerns about postmortem reactivation of virus. We analyzed multiple tissues for the presence of SVVspecific DNA and RNA. To study as many animals as possible virologically (while recognizing that statistical significance could not be achieved), we included eight monkeys that had been treated with short courses of different antiviral agents as presented in Table 1. The short course of antiviral treatments given to monkeys was part of different studies unrelated to the pathogenesis of SVV and was not an attempt by us to drive virus back into the latent state. In fact, none of the short antiviral treatments affected SVV transcription. Nevertheless, our model developed herein could still be used to determine if intravenous antiviral therapy followed by prolonged oral antiviral therapy might effectively reduce viral replication. For example, in humans with zoster sine herpete (radicular pain without rash caused by VZV), oral acyclovir failed to help patients, whereas intravenous acyclovir cured these patients and cleared their blood and cerebrospinal fluid of VZV (Gilden *et al*, 1994).

Previously, our laboratory had demonstrated the presence of SVV ORF 21-specific transcripts in pooled ganglia from 12 monkeys sacrificed 14 months after experimental infection (Clarke *et al*, 1996). Herein, we expanded this analysis to include more monkeys, more viral genes, more organs, and more intervals after infection. Individual animals were analyzed for SVV DNA and for SVV ORF 4-, 21-, 28-, 29-, 40-, 62-, and 63-specific transcripts, representing each gene class: IE, E, and L. SVV ORFs 21, 29, 62, and 63 are homologous to VZV-specific transcripts that have been detected in latently infected human ganglia (Cohrs *et al*, 1995, 1996). In addition to SVV-specific transcripts, sections of ganglia from two monkeys were analyzed for SVV-specific antigens.

SVV DNA sequences were detected in lung and liver up to 10 months p.i. and in all ganglia up to 12 months p.i. from SVV-infected animals, but not in lung, liver, and ganglia from an SVV-seronegative monkey. Since all TG and DRG from individual animals were pooled before nucleic acid extraction, while only small portions of each lung and liver were used, it is possible that lack of detection of SVV DNA in lung or liver 12 months p.i. reflects random sampling of viscera. We previously showed that most if not all of the SVV genome is present in ganglia and in adrenal gland years after experimental infection with SVV (Mahalingam et al, 1992, 1998). Interestingly, all regions of the SVV genome that were examined were detected in ganglia months after experimental infection; in contrast, not all regions of the SVV genome were present in some lung and liver samples at the same intervals. Thus, the SVV genome may be incomplete in these lung and liver samples. An incomplete alphaherpesvirus genome, that of herpes simplex virus (HSV), has been detected in nonganglionic sites such as brain years after primary infection (Fraser et al, 1981). Alternatively, the amount of SVV DNA in some lung and liver samples might have been below the limit of detection of the primer pairs used in the assay. Our results show that the lower limit of detection of the nested PCR used to amplify SVV ORF 4- and 63-specific DNA was 1 copy compared to 10 copies for SVV ORF 21-specific DNA. Thus, two of the three primer sets used in our PCR would detect a single copy of SVV DNA, and the third primer set would require 10 copies for detection.

We also showed that SVV is transcriptionally active in infected ganglia and other organs months after experimental infection. Nested PCR and RT-PCR were used because of their high level of sensitivity, thus ensuring that all negative samples were truly negative. Regarding RT-PCR, because oligodT primers were used to reverse transcribe mRNA, only polyadenylated transcripts would have been detected by our RT-PCR. Although no SVV-specific transcripts were detected in ganglia, lung, and liver RNA from an SVV-seronegative African green monkey, SVV-specific transcripts representing IE and E, but not L, gene classes were detected in ganglia from all monkeys 2 months p.i. SVV transcripts representing IE, putative E, and L gene classes were detected in all ganglia 5, 10, and 12 months p.i., respectively. Ganglia from one monkey 10 months p.i. revealed no SVV IE ORF 62-specific transcripts. Either ORF 62 was not transcribed in this ganglion or perhaps it was not polyadenylated and thus not detected after reverse transcription using an oligo(dT) primer. VZV ORF 62-specific transcripts expressed in latently infected human ganglia are not polyadenylated (Cohrs et al, 1996), and this also might be the case for SVV ORF 62 in some of the monkey ganglia.

IE, E, and L transcripts were detected in liver 2 and 5 months p.i., but not in lung from the same monkeys. In lung and liver, restricted viral transcription was observed 10 months p.i., with the detection of some IE and E transcripts, but not L transcripts. In one monkey sacrificed 10 months p.i., IE ORF 4 transcripts were detected in lung, but DNA representing ORF 4 was not detected. Although surprising, this could reflect a greater abundance of transcripts compared to the copy number of DNA below our limit of detection. No SVV DNA or SVV-specific transcripts were detected in lung and liver 12 months p.i. Limited transcription of other alphaherpesviruses in nonganglionic tissue has been reported. For example, the latency-associated transcripts (LATs) of herpes simplex virus have been found in cornea and brain (Cook et al, 1991; Kaye et al, 1991; Abghari et al, 1992; Lynas et al, 1993; Wharton et al, 1995), and bovine herpesvirus LATs were detected in lymphoreticular tissue of infected calves (Winkler et al, 2000) months after primary infection.

Our detection of SVV IE, E, and L transcripts in non-ganglionic tissue, a unique finding for varicella, prompted a search for SVV-specific proteins. We found SVV-specific antigen in ganglia and liver 10 days p.i., consistent with that reported by Dueland et al (1992). However, despite the detection of multiple SVV-specific transcripts in ganglia 12 months p.i., no SVV-specific proteins were detected in a SVV DNA-positive ganglion from the same monkey. In fact, no SVV-specific protein was detected in any ganglia examined 12 months p.i. While conclusions about the extent of SVV translation in monkeys months after experimental infection await analysis of additional ganglia, SVV-specific protein is either not present or is present at low abundance, consistent with the need to use nested RT-PCR to detect SVV-specific transcripts in ganglia at 12 months p.i. It is also possible that polyclonal anti-SVV IgG does not contain sufficient SVV-specific antibodies to detect low-abundance proteins in ganglia 12 months p.i. The use of SVV ORF-specific monoclonal or monospecific polyclonal antibodies may enhance the detection of SVV antigens in ganglia.

Because our monkeys were inoculated intratracheally with varicella virus, an exact comparison of our model to the pathogenesis of naturally occurring primary VZV infection in humans is impossible. Nonetheless, numerous virological differences are immediately obvious. First, in humans latently infected with VZV, only ganglia contain VZV compared to multiple organs of monkeys that contain SVV months after intratracheal inoculation. Second, the results of multiple investigations indicate that the extent of varicella transcription in latently infected humans appears to be limited to VZV genes 21, 29, 62, and 63, and translation is restricted to VZV gene 63 protein (Mahalingam et al, 1996; Kennedy et al, 2000). There is one outstanding, yet to be confirmed, report on the detection of VZV-specific proteins encoded by multiple viral genes in latently infected human ganglia (Lungu et al, 1998). Although further studies are necessary to determine the exact extent of varicella gene expression in ganglia, the most accurate comparison of VZV latency in humans to SVV in latency in monkeys will come from the study of tissues from monkeys with naturally acquired chickenpox.

Our only other study that experimentally infected monkeys with SVV both intratracheally and intravenously did reveal that multiple organs, including ganglia, became infected hematogenously before the rash of chickenpox developed (Mahalingam *et al*, 2001). However, those monkeys were sacrificed at earlier times after experimental infection, and no conclusion can be drawn about varicella latency to compare with the data from humans.

SVV has been recovered from viscera and on rare occasions from ganglia 2–10 days p.i. (Wenner *et al*, 1977; Iltis *et al*, 1982), but there are no reports of virus isolation from SVV-infected monkey tissues at later times. Because SVV has not been isolated from monkey tissue months after infection, and because the cell-associated nature of varicella virus prohibits its efficient isolation (Plotkin *et al*, 1977), we did not attempt to isolate SVV by cocultivation of ganglionic and nonganglionic cells with indicator cells. However, since our findings reveal a greater presence and transcription of virus than is seen during varicella latency in humans, our future studies will attempt to isolate virus as well as to identify virus particles by electron microscopy.

The detection of transcripts representing all gene classes, particularly L SVV transcripts, in multiple ganglia and viscera from most monkeys sacrificed 2– 12 months p.i. indicates possible viral DNA replication. The expression of most L herpesvirus genes is thought to depend on functional IE viral proteins and virus DNA replication. However, it is unknown if the extensive SVV-specific transcription observed in ganglia 2, 5, 10, and 12 months p.i. is regulated by viral proteins present at undetectable levels or by host cell-specific proteins. Furthermore, detection of SVV DNA and SVV-specific transcripts in lung or liver or both at multiple times p.i. indicates that SVV is transcriptionally active months after primary infection in nonganglionic tissues. Limited SVV transcription in these tissues may reflect a mechanism used by the virus to evade the host immune system. Alternatively, lung and liver cells that harbor SVV may be unable to support productive infection.

SVV transcription was limited to IE and E genes in ganglia of monkeys sacrificed 2 months p.i., which exhibited less severe rash and viremia during acute SVV infection compared to monkeys sacrificed 5, 10, and 12 months p.i., which expressed all three SVV gene classes. More animals inoculated under similar experimental conditions will need to be analyzed before further correlations can be made.

The discovery of SVV DNA and extensive viral transcription in ganglia and viscera months after experimental infection was unexpected. If SVV DNA had been found only in ganglia, and if viral gene expression had been restricted to 4-5 genes as in human ganglia latently infected with VZV, then two of the criteria for varicella latency would be satisfied. However, our detection of viral DNA and IE, E, and L transcripts in multiple tissues months after experimental infection indicates that this is not varicella latency. Our findings probably reflect the high virus load delivered intratracheally compared to naturally acquired varicella infection. To study SVV latency will require a careful virological examination of ganglia and other tissues from SVVseropositive monkeys months after naturally acquired infection.

Overall, the model described herein underlines the importance of route of inoculation and quantity of viral inoculum in determining the virological findings after experimental infection. In addition, our model of SVV infection has the potential to determine if treatment with antiviral agents, at a time when varicella gene expression is high, can clear tissue of virus.

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