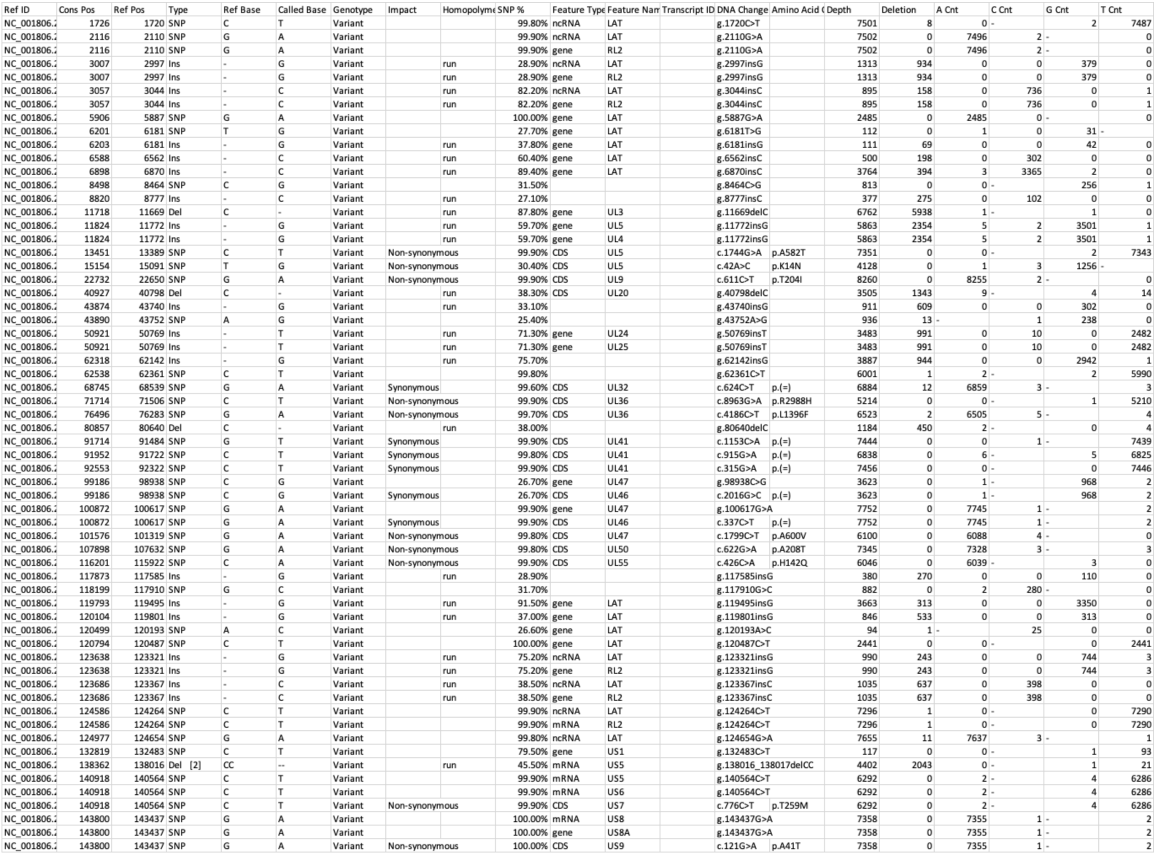
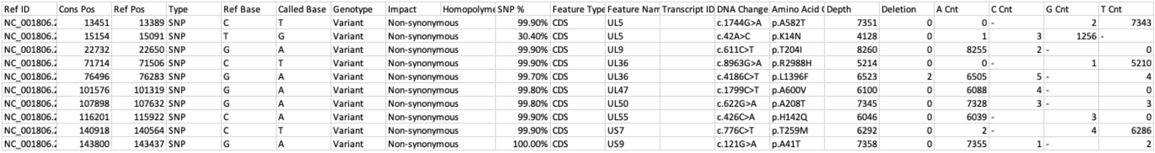
SUPPLEMENTARY INFORMATION

Supp. Table 1. All Putative SNPs. 17dmiR-H1/H6 was subject to Illumina sequencing and mapped to the HSV-1 17*syn*+ reference genome (GenBank NC_001806.2) using SeqMan NGen using default alignment parameters (merSize=21, merSkip=0, merSkipQuery=4 minMatchPercent=93, matchScore=10, mismatchPenalty=20, gapPenalty=50, gapExtensionPenalty=5, minAlignedLength=35, maxGap=20, autoTrim=true, filterDeepLayout=false, alignmentCutoff=20, gap5Prime=true, forceFullForwardAlign=false, DelayalignInserts=true, removeUniqueInserts=True, filterDeepLayoutOrganelle=True, removeDuplicateSeqs=false, combineDuplicateSeqs=True). Out of 6.0M paired reads, template coverage was 99.897% with median coverage of 6,797x. Variants were analyzed using SeqMan Ultra standard SNP filtering workflow (minDepth=5, minSnpFilter=25, pNotRef=90.0, pNotRefMinVal= 10.0). Variants were analyzed using SeqMan Ultra. The intended 25 bp deletion of mature miR-H1-5p/miR-H6-3p was observed at NC_001806.2 nt 8,021-8,045 and 118,329-118,353, representing the tRL and iRL segments respectively. 10 non-synonymous substitutions were observed but did not result in nonsense, no-start, no-stop, or frameshift mutations.



Ref ID	Cons Pos	Ref Pos	Type	Ref Base	Called Base	Genotype	Impact	Homopolyme	SNP %	Feature Type	Feature Nam	Transcript ID	DNA Change	Amino Acid (	Depth	Deletion	A Cnt	C Cnt	G Cnt	T Cnt
NC_001806.2	1726	1720	SNP	C	T	Variant			99.80%	ncRNA	LAT		g.1720C>T		7501	8	0	-	2	7487
NC_001806.2	2116	2110	SNP	G	A	Variant			99.90%	ncRNA	LAT		g.2110G>A		7502	0	7496	2	-	0
NC_001806.2	2116	2110	SNP	G	A	Variant			99.90%	gene	RL2		g.2110G>A		7502	0	7496	2	-	0
NC_001806.2	3007	2997	Ins	-	G	Variant		run	28.90%	ncRNA	LAT		g.2997insG		1313	934	0	0	379	0
NC_001806.2	3007	2997	Ins	-	G	Variant		run	28.90%	gene	RL2		g.2997insG		1313	934	0	0	379	0
NC_001806.2	3057	3044	Ins	-	C	Variant		run	82.20%	ncRNA	LAT		g.3044insC		895	158	0	736	0	1
NC_001806.2	3057	3044	Ins	-	C	Variant		run	82.20%	gene	RL2		g.3044insC		895	158	0	736	0	1
NC_001806.2	5906	5887	SNP	G	A	Variant			100.00%	gene	LAT		g.5887G>A		2485	0	2485	0	-	0
NC_001806.2	6201	6181	SNP	T	G	Variant			27.70%	gene	LAT		g.6181T>G		112	0	1	0	31	-
NC_001806.2	6203	6181	Ins	-	G	Variant		run	37.80%	gene	LAT		g.6181insG		111	69	0	0	42	0
NC_001806.2	6588	6562	Ins	-	C	Variant		run	60.40%	gene	LAT		g.6562insC		500	198	0	302	0	0
NC_001806.2	6898	6870	Ins	-	C	Variant		run	89.40%	gene	LAT		g.6870insC		3764	394	3	3365	2	0
NC_001806.2	8498	8464	SNP	C	G	Variant			31.50%				g.8464C>G		813	0	0	-	256	1
NC_001806.2	8820	8777	Ins	-	C	Variant		run	27.10%				g.8777insC		377	275	0	102	0	0
NC_001806.2	11718	11669	Del	C	-	Variant		run	87.80%	gene	UL3		g.11669delC		6762	5938	1	-	1	0
NC_001806.2	11824	11772	Ins	-	G	Variant		run	59.70%	gene	UL5		g.11772insG		5863	2354	5	2	3501	1
NC_001806.2	11824	11772	Ins	-	G	Variant		run	59.70%	gene	UL4		g.11772insG		5863	2354	5	2	3501	1
NC_001806.2	13451	13389	SNP	C	T	Variant	Non-synonymous		99.90%	CDS	UL5		c.1744G>A	p.A582T	7351	0	0	-	2	7343
NC_001806.2	15154	15091	SNP	T	G	Variant	Non-synonymous		30.40%	CDS	UL5		c.42A>C	p.K14N	4128	0	1	3	1256	-
NC_001806.2	22732	22650	SNP	G	A	Variant	Non-synonymous		99.90%	CDS	UL9		c.611C>T	p.T204I	8260	0	8255	2	-	0
NC_001806.2	40927	40798	Del	C	-	Variant		run	38.30%	CDS	UL20		g.40798delC		3505	1343	9	-	4	14
NC_001806.2	43874	43740	Ins	-	G	Variant		run	33.10%				g.43740insG		911	609	0	0	302	0
NC_001806.2	43890	43752	SNP	A	G	Variant			25.40%				g.43752A>G		936	13	-	1	238	0
NC_001806.2	50921	50769	Ins	-	T	Variant		run	71.30%	gene	UL24		g.50769insT		3483	991	0	10	0	2482
NC_001806.2	50921	50769	Ins	-	T	Variant		run	71.30%	gene	UL25		g.50769insT		3483	991	0	10	0	2482
NC_001806.2	62318	62142	Ins	-	G	Variant		run	75.70%				g.62142insG		3887	944	0	0	2942	1
NC_001806.2	62538	62361	SNP	C	T	Variant			99.80%				g.62361C>T		6001	1	2	-	2	5990
NC_001806.2	68745	68539	SNP	G	A	Variant	Synonymous		99.60%	CDS	UL32		c.624C>T	p.(=)	6884	12	6859	3	-	3
NC_001806.2	71714	71506	SNP	C	T	Variant	Non-synonymous		99.90%	CDS	UL36		c.8963G>A	p.R2988H	5214	0	0	-	1	5210
NC_001806.2	76496	76283	SNP	G	A	Variant	Non-synonymous		99.70%	CDS	UL36		c.4186C>T	p.L1396F	6523	2	6505	5	-	4
NC_001806.2	80857	80640	Del	C	-	Variant		run	38.00%				g.80640delC		1184	450	2	-	0	4
NC_001806.2	91714	91484	SNP	G	T	Variant	Synonymous		99.90%	CDS	UL41		c.1153C>A	p.(=)	7444	0	0	1	-	7439
NC_001806.2	91952	91722	SNP	C	T	Variant	Synonymous		99.80%	CDS	UL41		c.915G>A	p.(=)	6838	0	6	-	5	6825
NC_001806.2	92553	92322	SNP	C	T	Variant	Synonymous		99.90%	CDS	UL41		c.315G>A	p.(=)	7456	0	0	-	0	7446
NC_001806.2	99186	98938	SNP	C	G	Variant			26.70%	gene	UL47		g.98938C>G		3623	0	1	-	968	2
NC_001806.2	99186	98938	SNP	C	G	Variant	Synonymous		26.70%	CDS	UL46		c.2016G>C	p.(=)	3623	0	1	-	968	2
NC_001806.2	100872	100617	SNP	G	A	Variant			99.90%	gene	UL47		g.100617G>A		7752	0	7745	1	-	2
NC_001806.2	100872	100617	SNP	G	A	Variant	Synonymous		99.90%	CDS	UL46		c.337C>T	p.(=)	7752	0	7745	1	-	2
NC_001806.2	101576	101319	SNP	G	A	Variant	Non-synonymous		99.80%	CDS	UL47		c.1799C>T	p.A600V	6100	0	6088	4	-	0
NC_001806.2	107898	107632	SNP	G	A	Variant	Non-synonymous		99.80%	CDS	UL50		c.622G>A	p.A208T	7345	0	7328	3	-	3
NC_001806.2	116201	115922	SNP	C	A	Variant	Non-synonymous		99.90%	CDS	UL55		c.426C>A	p.H142Q	6046	0	6039	-	3	0
NC_001806.2	117873	117585	Ins	-	G	Variant		run	28.90%				g.117585insG		380	270	0	0	110	0
NC_001806.2	118199	117910	SNP	G	C	Variant			31.70%				g.117910G>C		882	0	2	280	-	0
NC_001806.2	119793	119495	Ins	-	G	Variant		run	91.50%	gene	LAT		g.119495insG		3663	313	0	0	3350	0
NC_001806.2	120104	119801	Ins	-	G	Variant		run	37.00%	gene	LAT		g.119801insG		846	533	0	0	313	0
NC_001806.2	120499	120193	SNP	A	C	Variant			26.60%	gene	LAT		g.120193A>C		94	1	-	25	0	0
NC_001806.2	120794	120487	SNP	C	T	Variant			100.00%	gene	LAT		g.120487C>T		2441	0	0	-	0	2441
NC_001806.2	123638	123321	Ins	-	G	Variant		run	75.20%	ncRNA	LAT		g.123321insG		990	243	0	0	744	3
NC_001806.2	123638	123321	Ins	-	G	Variant		run	75.20%	gene	RL2		g.123321insG		990	243	0	0	744	3
NC_001806.2	123686	123367	Ins	-	C	Variant		run	38.50%	ncRNA	LAT		g.123367insC		1035	637	0	398	0	0
NC_001806.2	123686	123367	Ins	-	C	Variant		run	38.50%	gene	RL2		g.123367insC		1035	637	0	398	0	0
NC_001806.2	124586	124264	SNP	C	T	Variant			99.90%	ncRNA	LAT		g.124264C>T		7296	1	0	-	0	7290
NC_001806.2	124586	124264	SNP	C	T	Variant			99.90%	mRNA	RL2		g.124264C>T		7296	1	0	-	0	7290
NC_001806.2	124977	124654	SNP	G	A	Variant			99.80%	ncRNA	LAT		g.124654G>A		7655	11	7637	3	-	1
NC_001806.2	132819	132483	SNP	C	T	Variant			79.50%	gene	US1		g.132483C>T		117	0	0	-	1	93
NC_001806.2	138362	138016	Del [2]	CC	--	Variant		run	45.50%	mRNA	US5		g.138016_138017delCC		4402	2043	0	-	1	21
NC_001806.2	140918	140564	SNP	C	T	Variant			99.90%	mRNA	US5		g.140564C>T		6292	0	2	-	4	6286
NC_001806.2	140918	140564	SNP	C	T	Variant			99.90%	mRNA	US6		g.140564C>T		6292	0	2	-	4	6286
NC_001806.2	140918	140564	SNP	C	T	Variant	Non-synonymous		99.90%	CDS	US7		c.776C>T	p.T259M	6292	0	2	-	4	6286
NC_001806.2	143800	143437	SNP	G	A	Variant			100.00%	mRNA	US8		g.143437G>A		7358	0	7355	1	-	2
NC_001806.2	143800	143437	SNP	G	A	Variant			100.00%	gene	US8A		g.143437G>A		7358	0	7355	1	-	2
NC_001806.2	143800	143437	SNP	G	A	Variant	Non-synonymous		100.00%	CDS	US9		c.121G>A	p.A41T	7358	0	7355	1	-	2

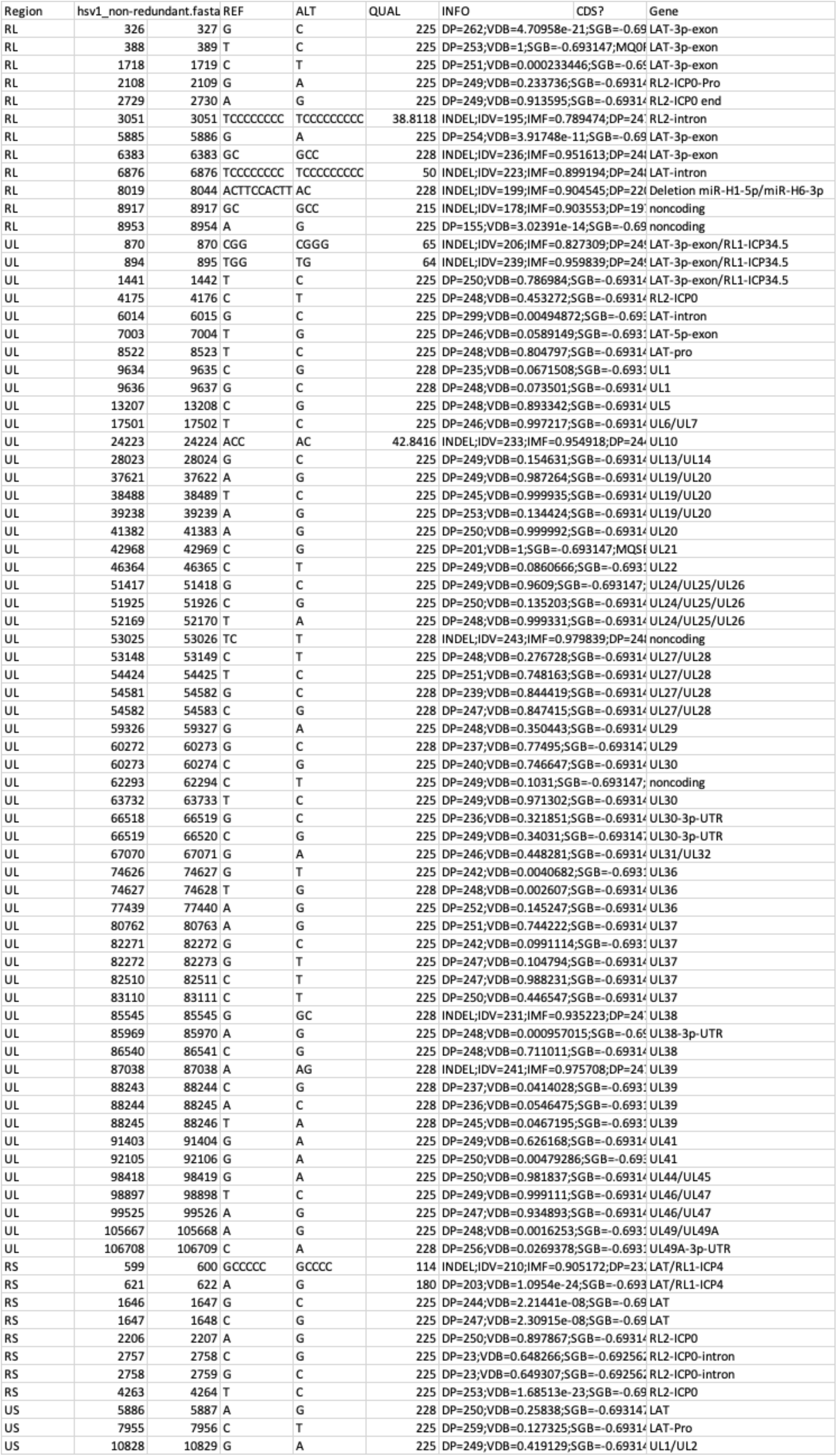
Supp. Table 2. Non-Synonymous SNPs. The impact of SNPs was inferred based on the GenBank NC_001806.2 reference. Of the putative SNPs 10 non-synonymous substitutions were observed using SeqMan Ultra.



Ref ID	Cons Pos	Ref Pos	Type	Ref Base	Called Base	Genotype	Impact	Homopolyme	SNP %	Feature Type	Feature Nam	Transcript ID	DNA Change	Amino Acid (	Depth	Deletion	A Cnt	C Cnt	G Cnt	T Cnt
NC_001806.2	13451	13389	SNP	C	T	Variant	Non-synonymous		99.90%	CDS	UL5		c.1744G>A	p.A582T	7351	0	0	-	2	7343
NC_001806.2	15154	15091	SNP	T	G	Variant	Non-synonymous		30.40%	CDS	UL5		c.42A>C	p.K14N	4128	0	1	3	1256	-
NC_001806.2	22732	22650	SNP	G	A	Variant	Non-synonymous		99.90%	CDS	UL9		c.611C>T	p.T204I	8260	0	8255	2	-	0
NC_001806.2	71714	71506	SNP	C	T	Variant	Non-synonymous		99.90%	CDS	UL36		c.8963G>A	p.R2988H	5214	0	0	-	1	5210
NC_001806.2	76496	76283	SNP	G	A	Variant	Non-synonymous		99.70%	CDS	UL36		c.4186C>T	p.L1396F	6523	2	6505	5	-	4
NC_001806.2	101576	101319	SNP	G	A	Variant	Non-synonymous		99.80%	CDS	UL47		c.1799C>T	p.A600V	6100	0	6088	4	-	0
NC_001806.2	107898	107632	SNP	G	A	Variant	Non-synonymous		99.80%	CDS	UL50		c.622G>A	p.A208T	7345	0	7328	3	-	3
NC_001806.2	116201	115922	SNP	C	A	Variant	Non-synonymous		99.90%	CDS	UL55		c.426C>A	p.H142Q	6046	0	6039	-	3	0
NC_001806.2	140918	140564	SNP	C	T	Variant	Non-synonymous		99.90%	CDS	US7		c.776C>T	p.T259M	6292	0	2	-	4	6286
NC_001806.2	143800	143437	SNP	G	A	Variant	Non-synonymous		100.00%	CDS	US9		c.121G>A	p.A41T	7358	0	7355	1	-	2

Supp. Table 3. BWA Variants. The 25 bp deletion was also confirmed using a standard SNP analysis workflow using BWA to aligning to a non-redundant HSV-1 genome where there only one copy of the repeat regions was retained(1) followed by variant calling with

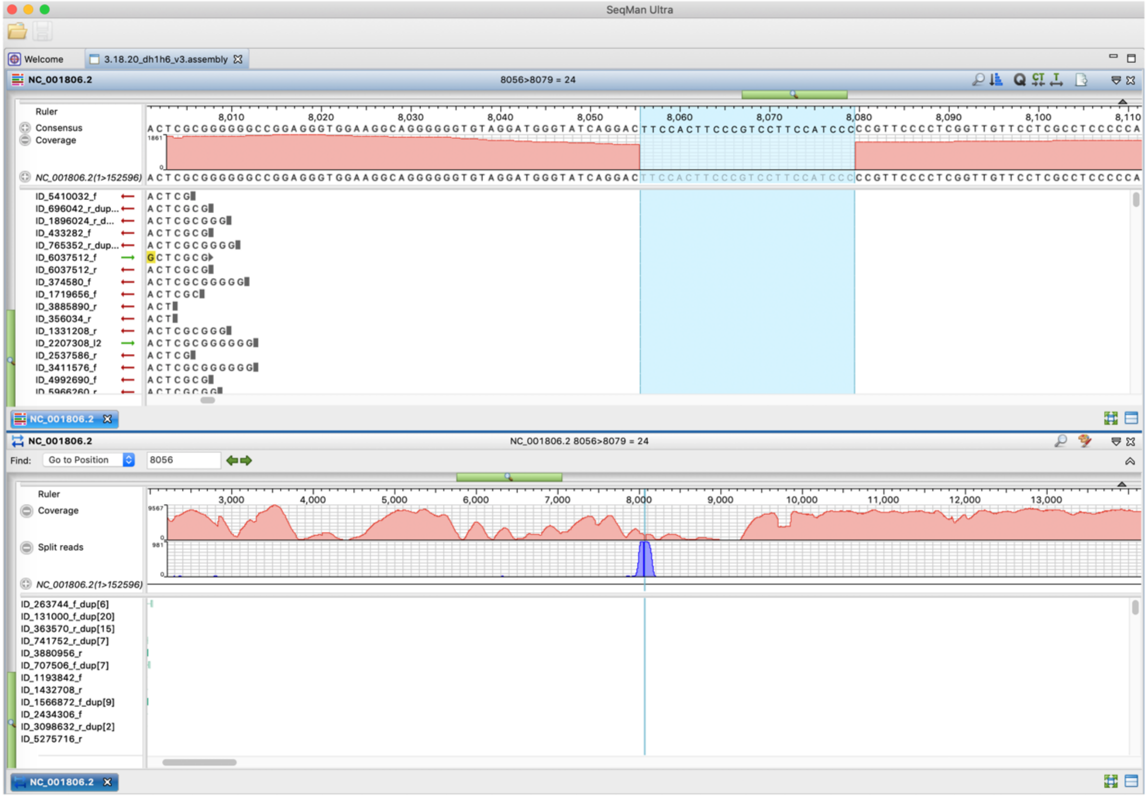
SAMtools and BCFtools, and visualization with Integrative Genomics Viewer. The in-house script is available from the authors upon request.



Region	hsv1_non-redundant.fasta		REF	ALT	QUAL	INFO	CDS?	Gene
RL	326	327	G	C	225	DP=262;VDB=4.70958e-21;SGB=-0.69		LAT-3p-exon
RL	388	389	T	C	225	DP=253;VDB=1;SGB=-0.693147;MQ0F		LAT-3p-exon
RL	1718	1719	C	T	225	DP=251;VDB=0.000233446;SGB=-0.69		LAT-3p-exon
RL	2108	2109	G	A	225	DP=249;VDB=0.233736;SGB=-0.69314		RL2-ICP0-Pro
RL	2729	2730	A	G	225	DP=249;VDB=0.913595;SGB=-0.69314		RL2-ICP0 end
RL	3051	3051	TCCCCCCCC	TCCCCCCCCC	38.8118	INDEL;IDV=195;IMF=0.789474;DP=24		RL2-intron
RL	5885	5886	G	A	225	DP=254;VDB=3.91748e-11;SGB=-0.69		LAT-3p-exon
RL	6383	6383	GC	GCC	228	INDEL;IDV=236;IMF=0.951613;DP=24		LAT-3p-exon
RL	6876	6876	TCCCCCCCC	TCCCCCCCCC	50	INDEL;IDV=223;IMF=0.899194;DP=24		LAT-intron
RL	8019	8044	ACTTCCACTT	AC	228	INDEL;IDV=199;IMF=0.904545;DP=22		Deletion miR-H1-5p/miR-H6-3p
RL	8917	8917	GC	GCC	215	INDEL;IDV=178;IMF=0.903553;DP=19		noncoding
RL	8953	8954	A	G	225	DP=155;VDB=3.02391e-14;SGB=-0.69		noncoding
UL	870	870	CGG	CGGG	65	INDEL;IDV=206;IMF=0.827309;DP=24		LAT-3p-exon/RL1-ICP34.5
UL	894	895	TGG	TG	64	INDEL;IDV=239;IMF=0.959839;DP=24		LAT-3p-exon/RL1-ICP34.5
UL	1441	1442	T	C	225	DP=250;VDB=0.786984;SGB=-0.69314		LAT-3p-exon/RL1-ICP34.5
UL	4175	4176	C	T	225	DP=248;VDB=0.453272;SGB=-0.69314		RL2-ICP0
UL	6014	6015	G	C	225	DP=299;VDB=0.00494872;SGB=-0.693		LAT-intron
UL	7003	7004	T	G	225	DP=246;VDB=0.0589149;SGB=-0.6931		LAT-5p-exon
UL	8522	8523	T	C	225	DP=248;VDB=0.804797;SGB=-0.69314		LAT-pro
UL	9634	9635	C	G	228	DP=235;VDB=0.0671508;SGB=-0.6931		UL1
UL	9636	9637	G	C	228	DP=248;VDB=0.073501;SGB=-0.69314		UL1
UL	13207	13208	C	G	225	DP=248;VDB=0.893342;SGB=-0.69314		UL5
UL	17501	17502	T	C	225	DP=246;VDB=0.997217;SGB=-0.69314		UL6/UL7
UL	24223	24224	ACC	AC	42.8416	INDEL;IDV=233;IMF=0.954918;DP=24		UL10
UL	28023	28024	G	C	225	DP=249;VDB=0.154631;SGB=-0.69314		UL13/UL14
UL	37621	37622	A	G	225	DP=249;VDB=0.987264;SGB=-0.69314		UL19/UL20
UL	38488	38489	T	C	225	DP=245;VDB=0.999935;SGB=-0.69314		UL19/UL20
UL	39238	39239	A	G	225	DP=253;VDB=0.134424;SGB=-0.69314		UL19/UL20
UL	41382	41383	A	G	225	DP=250;VDB=0.999992;SGB=-0.69314		UL20
UL	42968	42969	C	G	225	DP=201;VDB=1;SGB=-0.693147;MQSB		UL21
UL	46364	46365	C	T	225	DP=249;VDB=0.0860666;SGB=-0.6931		UL22
UL	51417	51418	G	C	225	DP=249;VDB=0.9609;SGB=-0.693147;		UL24/UL25/UL26
UL	51925	51926	C	G	225	DP=250;VDB=0.135203;SGB=-0.69314		UL24/UL25/UL26
UL	52169	52170	T	A	225	DP=248;VDB=0.999331;SGB=-0.69314		UL24/UL25/UL26
UL	53025	53026	TC	T	228	INDEL;IDV=243;IMF=0.979839;DP=24		noncoding
UL	53148	53149	C	T	225	DP=248;VDB=0.276728;SGB=-0.69314		UL27/UL28
UL	54424	54425	T	C	225	DP=251;VDB=0.748163;SGB=-0.69314		UL27/UL28
UL	54581	54582	G	C	228	DP=239;VDB=0.844419;SGB=-0.69314		UL27/UL28
UL	54582	54583	C	G	228	DP=247;VDB=0.847415;SGB=-0.69314		UL27/UL28
UL	59326	59327	G	A	225	DP=248;VDB=0.350443;SGB=-0.69314		UL29
UL	60272	60273	G	C	228	DP=237;VDB=0.77495;SGB=-0.693147		UL29
UL	60273	60274	C	G	225	DP=240;VDB=0.746647;SGB=-0.69314		UL30
UL	62293	62294	C	T	225	DP=249;VDB=0.1031;SGB=-0.693147;		noncoding
UL	63732	63733	T	C	225	DP=249;VDB=0.971302;SGB=-0.69314		UL30
UL	66518	66519	G	C	225	DP=236;VDB=0.321851;SGB=-0.69314		UL30-3p-UTR
UL	66519	66520	C	G	225	DP=249;VDB=0.34031;SGB=-0.693147		UL30-3p-UTR
UL	67070	67071	G	A	225	DP=246;VDB=0.448281;SGB=-0.69314		UL31/UL32
UL	74626	74627	G	T	225	DP=242;VDB=0.0040682;SGB=-0.6931		UL36
UL	74627	74628	T	G	228	DP=248;VDB=0.002607;SGB=-0.69314		UL36
UL	77439	77440	A	G	225	DP=252;VDB=0.145247;SGB=-0.69314		UL36
UL	80762	80763	A	G	225	DP=251;VDB=0.744222;SGB=-0.69314		UL37
UL	82271	82272	G	C	225	DP=242;VDB=0.0991114;SGB=-0.6931		UL37
UL	82272	82273	G	T	225	DP=247;VDB=0.104794;SGB=-0.69314		UL37
UL	82510	82511	C	T	225	DP=247;VDB=0.988231;SGB=-0.69314		UL37
UL	83110	83111	C	T	225	DP=250;VDB=0.446547;SGB=-0.69314		UL37
UL	85545	85545	G	GC	228	INDEL;IDV=231;IMF=0.935223;DP=24		UL38
UL	85969	85970	A	G	225	DP=248;VDB=0.000957015;SGB=-0.69		UL38-3p-UTR
UL	86540	86541	C	G	225	DP=248;VDB=0.711011;SGB=-0.69314		UL38
UL	87038	87038	A	AG	228	INDEL;IDV=241;IMF=0.975708;DP=24		UL39
UL	88243	88244	C	G	228	DP=237;VDB=0.0414028;SGB=-0.6931		UL39
UL	88244	88245	A	C	228	DP=236;VDB=0.0546475;SGB=-0.6931		UL39
UL	88245	88246	T	A	228	DP=245;VDB=0.0467195;SGB=-0.6931		UL39
UL	91403	91404	G	A	225	DP=249;VDB=0.626168;SGB=-0.69314		UL41
UL	92105	92106	G	A	225	DP=250;VDB=0.00479286;SGB=-0.693		UL41
UL	98418	98419	G	A	225	DP=250;VDB=0.981837;SGB=-0.69314		UL44/UL45
UL	98897	98898	T	C	225	DP=249;VDB=0.999111;SGB=-0.69314		UL46/UL47
UL	99525	99526	A	G	225	DP=247;VDB=0.934893;SGB=-0.69314		UL46/UL47
UL	105667	105668	A	G	225	DP=248;VDB=0.0016253;SGB=-0.6931		UL49/UL49A
UL	106708	106709	C	A	228	DP=256;VDB=0.0269378;SGB=-0.6931		UL49A-3p-UTR
RS	599	600	GCCCCC	GCCCC	114	INDEL;IDV=210;IMF=0.905172;DP=23		LAT/RL1-ICP4
RS	621	622	A	G	180	DP=203;VDB=1.0954e-24;SGB=-0.693		LAT/RL1-ICP4
RS	1646	1647	G	C	225	DP=244;VDB=2.21441e-08;SGB=-0.69		LAT
RS	1647	1648	C	G	225	DP=247;VDB=2.30915e-08;SGB=-0.69		LAT
RS	2206	2207	A	G	225	DP=250;VDB=0.897867;SGB=-0.69314		RL2-ICP0
RS	2757	2758	C	G	225	DP=23;VDB=0.648266;SGB=-0.692562		RL2-ICP0-intron
RS	2758	2759	G	C	225	DP=23;VDB=0.649307;SGB=-0.692562		RL2-ICP0-intron
RS	4263	4264	T	C	225	DP=253;VDB=1.68513e-23;SGB=-0.69		RL2-ICP0
US	5886	5887	A	G	228	DP=250;VDB=0.25838;SGB=-0.693147		LAT
US	7955	7956	C	T	225	DP=259;VDB=0.127325;SGB=-0.69314		LAT-Pro
US	10828	10829	G	A	225	DP=249;VDB=0.419129;SGB=-0.69314		UL1/UL2

A

tRL miR-H1/H6 Sequence



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Consensus              ACTCGCGGGGGGCCGGAGGGTGGAAGGCAGGGGGGTGTAGGATGGGTATCAGGACTTCCACTTCCCGTCCTTCCATCCCCCGTTCCCCTCGGTTGTTCCTCGCCTCCCCA
NC_001806.2(1>152596)  ACTCGCGGGGGGCCGGAGGGTGGAAGGCAGGGGGGTGTAGGATGGGTATCAGGACTTCCACTTCCCGTCCTTCCATCCCCCGTTCCCCTCGGTTGTTCCTCGCCTCCCCA

ID_5410032_f       ←  ACTCG
ID_696042_r_dup... ←  ACTCGCG
ID_1896024_r_d...  ←  ACTCGCGGG
ID_433282_f        ←  ACTCGCG
ID_765352_r_dup... ←  ACTCGCGGGG
ID_6037512_f       →  GCTCGCG
ID_6037512_r       ←  ACTCGCG
ID_374580_f        ←  ACTCGCGGGGG
ID_1719656_f       ←  ACTCGC
ID_3885890_r       ←  ACT
ID_356034_r        ←  ACT
ID_1331208_r       ←  ACTCGCGGG
ID_2207308_l2      →  ACTCGCGGGGGG
ID_2537586_r       ←  ACTCG
ID_3411576_f       ←  ACTCGCGGGGGG
ID_4992690_f       ←  ACTCGCG
ID_5966260_r       ←  ACTCGCGG

ID_263744_f_dup[6]
ID_131000_f_dup[20]
ID_363570_r_dup[15]
ID_741752_r_dup[7]
ID_3880956_r
ID_707506_f_dup[7]
ID_1193842_f
ID_1432708_r
ID_1566872_f_dup[9]
ID_2434306_f
ID_3098632_r_dup[2]
ID_5275716_r
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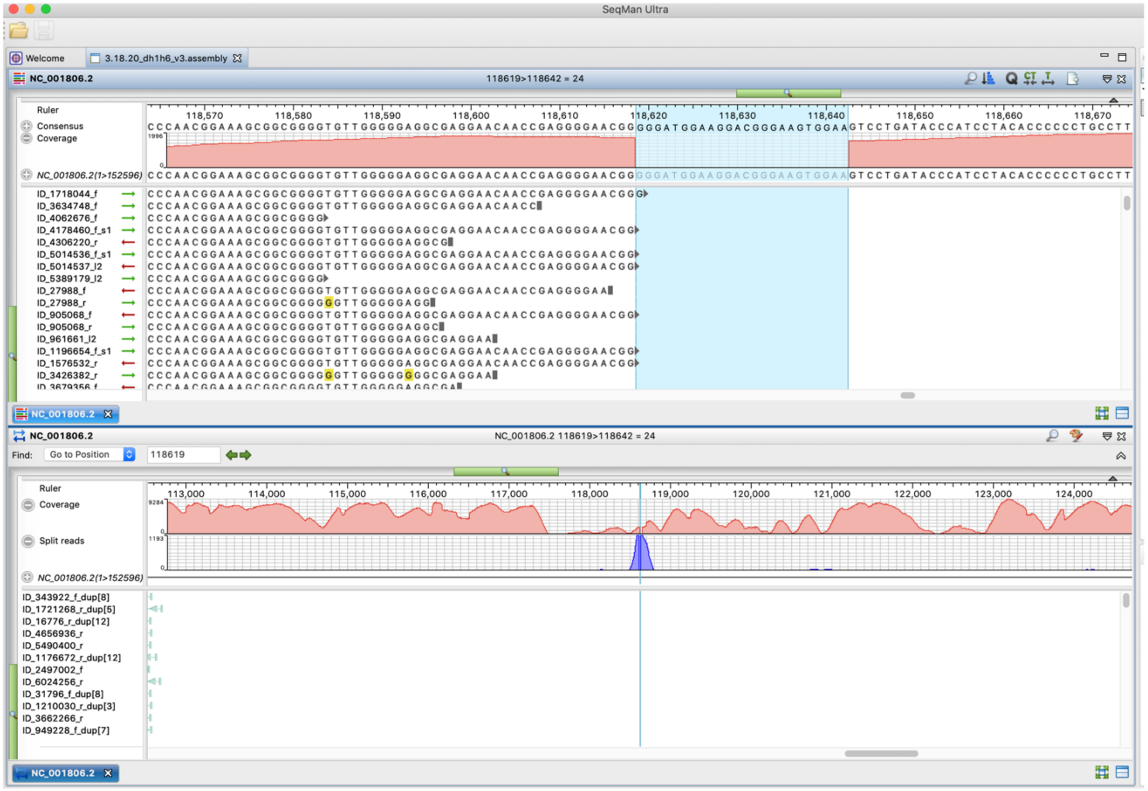
B

iRL miR-H1/H6 Sequence



C

tRL miR-H1/H6 Sequence Non-redundant Reference

Supp. Figure 1. Confirmation of miR-H1/H6 Deletion. 17dmiR-H1/H6 was subject to Illumina sequencing and (**A**-**B**) mapped to the HSV-1 17*syn*+ reference genome (GenBank NC_001806.2) using SeqMan NGen. Variants were analyzed using SeqMan Ultra. The intended 25 bp deletion of mature miR-H1-5p/miR-H6-3p was observed at NC_001806.2 nt 8,021-8,045 and 118,329-118,353, representing the(**A**) tRL and (**B**) iRL segments respectively. **C**) The 25 bp deletion was also confirmed using a standard SNP analysis workflow using BWA to aligning to a non-redundant HSV-1 genome where there only one copy of the repeat regions was retained(1) followed by variant calling with SAMtools and BCFtools, and visualization with Integrative Genomics Viewer.

Supplementary References.

1. Morse AM, Calabro KR, Fear JM, Bloom DC, McIntyre LM. 2017. Reliable Detection of Herpes Simplex Virus Sequence Variation by High-Throughput Resequencing. Viruses 9:226.