Genotypic and phenotypic diversity within the neonatal HSV-2 population

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18 # Abstract

19 More than 14,000 neonates are infected with herpes simplex virus (HSV) annually.
20 Approximately half display manifestations limited to the skin, eyes, or mouth (SEM disease).
21 The rest develop invasive infections that spread to the central nervous system (CNS disease or
22 encephalitis) or systemically (disseminated disease). Invasive HSV disease is associated with
23 significant morbidity and mortality, but viral and host factors that predispose neonates to these
24 forms are unknown. To define viral diversity within the infected neonatal population, we
25 evaluated ten HSV-2 isolates from newborns with a range of clinical presentations. To assess
26 viral fitness independent of host immune factors, we measured viral growth characteristics in
27 cultured cells and found diverse *in vitro* phenotypes. Isolates from neonates with CNS disease
28 were associated with larger plaque size and enhanced spread, with isolates from cerebrospinal
29 fluid (CSF) exhibiting the most robust growth. We sequenced complete viral genomes of all ten
30 neonatal viruses, providing new insights into HSV-2 genomic diversity in this clinical setting.
31 We found extensive inter-host and intra-host genomic diversity throughout the viral genome,
32 including amino acid differences in more than 90% of the viral proteome. The genes encoding
33 glycoprotein G (gG, US4), gI (US7), gK (UL53), and viral proteins UL8, UL20, UL24, and US2
34 contained variants that were found in association with CNS isolates. Many of these viral proteins
35 are known to contribute to cell spread and neurovirulence in mouse models of CNS disease. This
36 study represents the first application of comparative pathogen genomics to neonatal HSV
37 disease.

38

39 # Importance

40 Herpes simplex virus (HSV) causes invasive disease in half of infected neonates, resulting in
41 significant mortality and permanent cognitive morbidity. The factors that contribute to invasive
42 disease are not understood. This study reveals diversity among HSV isolates from infected
43 neonates, and makes the first associations between viral genetic variations and clinical disease
44 manifestations. We found that viruses isolated from newborns with encephalitis show enhanced
45 spread in culture. These viruses contain protein-coding variations not found in viruses causing
46 non-invasive disease. Many of these variations are found in proteins known to impact
47 neurovirulence and viral spread between cells. This work advances our understanding of HSV
48 diversity in the neonatal population and how it may impact disease outcome.

49

50 # Introduction

51 Each year an estimated 10,000 neonates are infected with HSV-2, and 4,000 infected with HSV-
52 1, worldwide (1). Infants are typically infected at the time of birth by maternal genital shedding
53 of HSV, most often by mothers who are not aware of their infection (2–4). The recent increase in
54 genital HSV-1 incidence among women of childbearing age, particularly in developed nations,
55 suggests that the burden of neonatal infection will continue to rise (1, 5). While some infected
56 infants exhibit only superficial infection limited to the skin, eyes, or mouth (SEM disease; 45%),
57 about half develop invasive systemic (disseminated disease; 25%) or central nervous system
58 (CNS disease; 30%) infections associated with significant morbidity and mortality (6, 7).
59 Currently, the antiviral medication acyclovir is the standard therapy for all forms of neonatal

60 HSV disease. Although this intervention has reduced mortality due to invasive disease, most
61 survivors of invasive disease are left with permanent neurodevelopmental deficits (8, 9).

62 The factors that predispose a neonate to invasive HSV infection are not entirely known. Recent
63 studies have found that some adults and children outside of the neonatal period who experience
64 HSV infection of the brain have a host genetic defect within the Toll-like receptor-3 (TLR3)
65 pathway (10, 11). Outside of the neonatal period, HSV encephalitis is rare, as are host defects in
66 the TLR3-pathway. By contrast, half of HSV-infected neonates experience invasive CNS or
67 disseminated disease, making it less likely that host genetic defects alone could account for all of
68 the observed cases of invasive infection in neonates. Prior clinical data on mother-to-infant
69 transmission of HSV indicate that most cases of neonatal disease, including invasive forms of
70 disease, result from newly acquired or primary HSV infection prior to the development of
71 maternal antibody production (2–4, 12). This suggests a window of opportunity where the
72 contributions of viral genetic variation to the progression of invasive infection and disease may
73 be greater than in adults.

74 Prior studies have identified viral genetic factors that influence virulence or disease for
75 reoviruses, influenza virus, HIV, and others (13–18). In contrast to these RNA viruses, HSV was
76 presumed to have lower genetic diversity and potential for variation in virulence, due to its
77 relatively stable DNA genome and long co-evolutionary history with humans (19). The
78 assumption of limited HSV heterogeneity was supported by early studies that utilized low-
79 resolution restriction fragment length polymorphism (RFLP) or single-gene analyses to compare
80 multiple HSV isolates (20–22). However Rosenthal and colleagues used RFLP and PCR analysis
81 of a single locus to demonstrate that a heterogeneous HSV population can exist in an invasive

82 neonatal infection, and provided proof of principle that natural genetic variation can impact
83 neurovirulence (23, 24). More recently, advances in high-throughput sequencing (HTSeq) have
84 enabled a re-evaluation of herpesvirus genome-wide variation, which suggests that herpesviruses
85 harbor extensive diversity both between strains or individuals (inter-host variation) as well as
86 within a single individual (intra-host variation) (25–27). These minor genetic variants may
87 become clinically important if a variant within the viral population becomes the new dominant
88 allele or genotype as a result of a bottleneck at transmission, entry into a new body compartment,
89 or selective pressure such as antiviral therapy (28, 29).

90 Several recent examples have demonstrated the potential for new insights gained by applying
91 HTSeq approaches to herpesvirus infections in a clinical setting. In HTSeq studies of congenital
92 infection by the beta-herpesvirus human cytomegalovirus (HCMV), Renzette *et al.* found
93 evidence for heterogeneous viral populations both within and between hosts (30–35). The levels
94 of diversity observed in congenital HCMV infections far exceeded those observed in adult
95 infections (36–38). HTSeq-based examination of vaccine-associated rashes due to the alpha-
96 herpesvirus varicella zoster virus (VZV) demonstrated that adult skin vesicles contain a subset of
97 the viral population introduced during vaccination, and found at least 11 VZV genomic loci that
98 were linked to rash formation (26). Recent HTSeq comparisons of adult genital HSV-2 revealed
99 the first evidence of a single individual shedding two distinct strains (39), demonstrated changes
100 in the viral genome over time in a recently infected host (40), and provided the first evidence of
101 ancient recombination between HSV-1 and HSV-2 (41, 42). However, to date there has been no
102 evaluation of genome-wide variation in neonatal HSV isolates to determine the levels of
103 diversity in this population, or the potential impact(s) of viral genetic variants on disease.

104 Until recently, several technical barriers prevented thorough assessment of neonatal HSV
105 genomes. A key constraint on studies of neonatal disease has been the availability of cultured,
106 minimally-passaged viral samples that are both associated with clinical information and have
107 also been maintained in a low-passage state that is appropriate for sequencing and further
108 experimental studies. Historically viral culture was part of the HSV diagnostic workflow, but this
109 has been superseded in clinical laboratory settings by the speed and sensitivity of viral detection
110 by PCR (6, 43, 44). This change limits neonatal HSV sample availability for *in vitro* and animal
111 model studies. In addition, many previously archived neonatal HSV isolates have been passaged
112 extensively, allowing them to acquire mutations that enhance viral growth in culture (23, 24).
113 Additional challenges for HTSeq approaches to neonatal HSV include the large size of the viral
114 genome (~152 kb), its high G+C content (~70%), and a large number of variable-number tandem
115 repeats in the viral genome (> 240 mini/micro-satellite repeats and >660 homopolymers of ≥6
116 base pairs) (27, 45). Therefore, many studies of HSV diversity (21, 46–49) or the effect of HSV
117 genetic variation on disease (50–52) have relied on low-resolution restriction fragment length
118 polymorphism (RFLP) or single-gene PCR analyses, due to the speed and ease of analysis in
119 comparison to whole-genome approaches (53–58). To overcome these challenges, we combined
120 our expertise in HSV comparative genomics and phenotypic analysis (53, 58–60) with a unique
121 resource of low-passage, well-annotated neonatal specimens (8, 9, 61).

122 Here we analyzed a set of ten low-passage clinical HSV-2 isolates collected from neonates with
123 HSV infection, enrolled in one of two clinical studies that spanned three decades of patient
124 enrollment (1981-2008) (8, 9, 61). These samples represented a wide range of clinical
125 manifestations including SEM, CNS, and disseminated disease, and each sample was associated
126 with de-identified clinical information. We defined the level of diversity in this population using

127 comparative genomics and an array of cell-based phenotypic assays. We found that HSV-2
128 isolates displayed diverse *in vitro* phenotypes, as well as extensive inter- and intra-host diversity
129 distributed throughout the HSV-2 genome. Finally, we found coding variations in several HSV-2
130 proteins associated with CNS disease. This study represents the first-ever application of
131 comparative pathogen genomics to neonatal HSV disease and provides a basis for further
132 exploration of genotype-phenotype links in this clinically vulnerable patient population.

133 Results

134 Neonatal HSV-2 samples represent a diverse clinical population

135 We utilized samples collected from ten HSV-2-infected neonates enrolled by the National
136 Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (CASG) for
137 clinical trials between 1981 and 2008 (8, 9, 61). These infants encompassed a range of clinical
138 disease manifestations (see **Table 1**), with about half experiencing invasive CNS disease (5
139 patients) or disseminated (DISS) disease with CNS involvement (2 patients), and the remainder
140 experiencing non-invasive SEM disease (3 patients). Extensive clinical information was
141 available for each patient, including long-term neurocognitive and motor outcomes (**Table 1**).
142 This population was also diverse with respect to sex, race, gestational age, and enrollment center
143 (**Table 1**; enrollment center data not shown). All samples were collected at the time of diagnosis,
144 prior to initiation of acyclovir therapy. Each isolate was cultured once as part of the diagnostic
145 process, with expansion only for the experiments shown here. Although the sample size was
146 constrained by the rarity of neonatal HSV infection and availability of appropriately maintained
147 isolates, our group is similar in size to prior HTSeq comparisons of congenital HCMV samples

148 (30–35), and is the largest group of neonatal HSV samples ever subjected to comparative
149 genomic and phenotypic analysis.

150 Neonatal HSV-2 isolates have different fitness in culture

151 To determine whether the viruses isolated from this neonatal population **(Table 1)** were
152 intrinsically different, we assessed viral growth in culture, which provides a consistent
153 environment that is independent of host genetic variation. To minimize the impact of immune
154 pressure we selected Vero monkey kidney cells, which lack an interferon response (62, 63). Each
155 viral isolate was applied to a confluent monolayer of cells *in vitro*, and allowed to form plaques
156 for 100 hours (h) (**Figure 1A**). Average plaque size differed between isolates, with six of the ten
157 isolates being statistically larger (indicated in green) than the other four (indicated in black,
158 **Figure 1B**) (one-way ANOVA with Holm-Sidak's multiple comparisons test, $p<0.05$). The
159 average plaque size of the previously described low passage, adult HSV-2 isolate SD90e is
160 shown for comparison (64). The largest plaque sizes were observed in the two viruses isolated
161 directly from the cerebrospinal fluid (CSF; isolates CNS11 and DISS14; **Figure 1B**); these two
162 isolates were not statistically different from one another in size, but were significantly larger than
163 any of those isolated from the skin (one-way ANOVA with Holm-Sidak's multiple comparisons
164 test, $p<0.05$). Plaque size was assessed at each passage in culture and remained constant from the
165 time the isolates were received in our laboratory (passage 2) through their genetic and
166 phenotypic analysis (passage 4). The variance in plaque sizes produced by a given isolate was
167 not statistically different between isolates **(Figure 1B)**. The differences in average plaque size
168 between isolates suggested that the HSV-2 populations found in each neonatal isolate are indeed
169 intrinsically different.

170 **Entry kinetics, DNA replication, protein expression, and virus production do not**
171 **account for differences in plaque size**

172 Plaque formation is a complex endpoint that involves the ability of the virus to enter the cell,
173 replicate its double stranded DNA genome, produce viral proteins and assemble new virions that
174 spread to adjacent cells. Therefore, we explored whether the differences in plaque formation
175 observed in Vero cells reflected inherent differences in the ability of isolates to complete each of
176 these stages of the viral life cycle. For these comparisons, two large-plaque-forming isolates
177 (CNS11 and CNS03) were compared to two small-plaque-forming isolates (CNS12 and SEM02).
178 First, we compared each isolate's rate of cell entry. Virus was applied to chilled cells, followed
179 by warming to synchronize cell entry **(Figure 2A).** A low pH solution was applied at various
180 points over the first hour of cell entry to inactivate any virus that had not yet entered a cell, and
181 plaque formation was then allowed to proceed for 100 h. We found no difference in rates of cell
182 entry between these four representative viral isolates (**Figure 2B**), suggesting that large plaques
183 did not result from increased rates of virus entry into cells.

184 We next infected Vero cells at high multiplicity of infection (MOI=5) to compare the outcome of
185 a single round of viral replication. We found that all four isolates produced similar numbers of
186 genome copies (as measured by qPCR for the gB gene; see Methods for details) **(Figure 2C**),
187 indicating that differences in viral DNA replication did not influence plaque size. We quantified
188 the production of infectious virus by counting plaque-forming-units (PFU) on Vero cell
189 monolayers, as well as on the highly-permissive U2OS human bone osteosarcoma epithelial cell
190 line (65). No differences in virus production were noted between the four isolates when
191 quantified on either cell type (**Figure 2D, E**). U2OS cells lack innate sensing of viral infection

192 through the STING pathway (66) and can even support the growth of highly-defective HSV
193 isolates that lack ICP0 function (65). All isolates formed large plaques on the highly-permissive
194 U2OS cell monolayers (**Figure S1**), allowing us to rule out the possibility that very small foci of
195 infection were missed during titering of the small plaque-forming isolates on Vero cells (**Figure**
196 **2D, E**). Finally, we compared viral protein production for these isolates, and found no
197 differences in the expression levels of a panel of HSV-2 viral proteins at either early (6 h post
198 infection, hpi) or late (24 hpi) time points of a single round of high MOI infection (**Figure 2F,**
199 **G**; see legend for list of proteins). Taken together, these results suggested that large- and small-
200 plaque-forming neonatal HSV-2 isolates did not differ significantly in viral entry, DNA
201 replication, infectious virus production, or protein production over a single round of infection.

202 Large-plaque-forming isolates exhibit enhanced cell-to-cell spread

203 We next assessed the ability of representative large- and small-plaque-forming isolates to spread
204 from cell-to-cell. Vero cell monolayers were infected at low MOI (MOI=0.001) in order to assess
205 differences over multiple rounds of viral replication and spread throughout the cell monolayer
206 (**Figure 3A**). The contribution of indirect cell-free spread was minimized by including 0.1%
207 human serum in the media, and changing the media every 24 h to remove released virus and
208 refresh serum levels. Over a 72-hour time course, cell monolayers were assessed to measure the
209 extent of cell-to-cell spread, either by harvesting and titering PFU production (**Figure 3B, C**), or
210 by fixing infected cell monolayers and evaluating the distribution of virus by
211 immunofluorescence (**Figure 3D**, **E**, and **Figure S2**). Viral titers recovered from harvested cells
212 were similar at 2 hpi, confirming that equivalent amounts of each virus were present following
213 initial infection (**Figure 3B**). By 72 hpi, after multiple rounds of replication and spread, the

214 large-plaque-forming isolates CNS11 and CNS03 had achieved viral titers significantly greater
215 than those of the small-plaque-forming isolates CNS12 and SEM02 (**Figure 3B, C**; two-way
216 ANOVA followed by Tukey's multiple comparison test, $p<0.0001$ at 72h). Isolate CNS11,
217 which was obtained directly from the CSF, produced titers statistically greater than the other
218 three isolates **(Figure 3B, C)**.

219 We also directly evaluated cell-to-cell spread by immunostaining and quantifying the distribution
220 of infected cells around each infectious focus. Infected Vero cell monolayers were fixed at 24,
221 48, and 72 hpi, and subjected to fluorescent immunocytochemistry with an antibody directed
222 against total HSV (**Figure 3D** and **Figure S2**). The region of HSV-positive cells surrounding a
223 single initial infection was greater for the large-plaque-forming neonatal HSV-2 isolates at 24
224 hpi, and increased by 48 and 72 hpi. The central cytolytic clearings seen in the monolayers
225 infected by CNS11 or CNS03 were approximately two-fold greater than those infected by
226 CNS12 or SEM02, reflecting the average two-fold increase in plaque size observed in
227 methylene-blue-stained monolayers in **Figure 1**. However, the region of infected cells
228 surrounding the central cytolytic clearing for CNS11 and CNS03 was dramatically larger than
229 for CNS12 and SEM02, suggesting that large-plaque-forming neonatal HSV-2 isolates show
230 greater spread from cell-to-cell than could have been predicted by measuring plaque size alone.
231 To quantify this increase in the area of infected cells after a low-MOI infection, each coverslip
232 was imaged **(Figure S2)** and the total number of immunofluorescent pixels was quantified
233 (**Figure 3E**). By 72 hpi, the area of HSV-infected cells was statistically greater for the large-
234 plaque-forming isolates CNS11 and CNS03, as compared to the small-plaque-forming isolates
235 CNS12 and SEM02 (two-way ANOVA followed by Tukey's multiple comparison test, $p<0.05$ at
236 72h). Together these data indicated that large-plaque-forming isolates shared an enhanced ability

237 to spread cell-to-cell through culture, in comparison to small-plaque-forming isolates.

238 Comparative genomics reveals genetic diversity in neonatal HSV-2 isolates

239 The differences identified in cell-to-cell spread between neonatal isolates in culture indicated the
240 existence of intrinsic differences between these viruses. To reveal how genetic variation may
241 contribute to viral phenotypes in culture, and ultimately to clinical disease manifestations, we
242 sequenced the complete viral genome of all ten neonatal HSV-2 isolates. For each isolate, we
243 sequenced purified viral nucleocapsid DNA and assembled a consensus genome, which
244 represents the most common genotype at each nucleotide locus in the viral population. The
245 clinical trials utilized in this study enrolled HSV-infected infants from multiple sites across the
246 United States (8, 9, 61). Therefore, we first assessed the overall degree of relatedness between
247 these viral genomes to understand whether any similarities in viral or geographic origin might
248 have contributed to *in vitro* or clinical phenotype patterns. In light of the known potential for
249 recombination in the phylogenetic history of HSV (49, 67, 53, 42), we used a graph-based
250 network to investigate the phylogenetic relationship between these isolates. We found a similar
251 degree of divergence among all ten neonatal HSV-2 consensus genomes (**Figure 4A**). We then
252 compared these ten neonatal HSV-2 genomes to all available HSV-2 genomes in GenBank (all of
253 which are adult; see **Table S1** for HSV-2 GenBank accessions and references) to discern any
254 geographic or other clustering (see **Table 1**). The neonatal isolates did not segregate into any
255 exclusive groupings, but instead intermingled among non-neonatal isolates, with deep branches
256 between each isolate (**Figure 4B**). These findings were corroborated using an alternative
257 phylogenetic clustering algorithm as well (**Figure S3**). This suggested that similarities in viral
258 genetic origin were not responsible for determining the cellular or clinical outcomes of neonatal

259 HSV-2 infection.

260 Overall protein-coding diversity in neonatal HSV-2 isolates is similar to that 261 observed in adult HSV-2 strains

262 We next asked whether overt defects in any single HSV-2 protein might be associated with
263 clinical or *in vitro* spread phenotypes. In examining the coding potential of all ten neonatal HSV-
264 2 isolates, we found no protein deletions or truncations encoded by any of these viral genomes.
265 These comparisons revealed a total of 784 nucleotide differences in 71 genes (see **Table S2**),
266 resulting in 342 non-synonymous AA differences in 65 proteins. This led to an average of 1%
267 coding variation (range 0.0 : 2.6%). This variation was spread widely throughout the HSV-2
268 genome, without concentration in a particular genomic region or category of protein function
269 **(Figure 5).** We found a similar level of coding diversity in this set of neonatal isolates as in a
270 comparable set of 10 adult HSV-2 isolates **(Figure 5, Table S2).** We also compared these
271 neonatal genomes to the full set of 58 annotated adult HSV-2 genomes from GenBank (listed
272 **Table S1**). As expected from the difference in sample size, there was more overall diversity
273 found across 58 adult HSV-2 genomes than in 10 neonatal genomes. A comparison of the dN/dS
274 ratio in neonatal vs. adult HSV-2 genomes revealed a similar trend with a few outliers visible on
275 each axis, e.g. UL38 and US8A had a higher dN/dS ratio in neonatal than in adult isolates (see
276 **Table S2** and **Figure S4** for full comparison). These data indicated that at the consensus level,
277 neonatal HSV-2 isolates display substantial inter-host coding diversity spread throughout the
278 genome, but do not possess strikingly more diversity or an excess of genetic drift as compared to
279 adult isolates.

280 **Minor variants expand the potential coding diversity of neonatal HSV-2 isolates**

281 We next focused our attention on differences below the consensus level in each intra-host viral
282 population. The amino acid (AA) variations described above exist in the consensus genomes of
283 each isolate. Since viral replication creates a population of genomes, we next assessed whether
284 minor allelic variants existed within the viral population of any neonatal HSV-2 isolate, thereby
285 expanding the viral genetic diversity within each host. The significant depth of coverage from
286 deep-sequencing of each isolate allowed us to search for minor variants at every nucleotide
287 position of each genome. We defined a minor variant as any nucleotide allele (single nucleotide
288 polymorphism, or SNP) or insertion/deletion (INDEL) with frequency below 50%, but above 2%
289 (a conservative limit of detection; see Methods for additional criteria). We found minor variants
290 in the viral genome population of all 10 neonatal HSV-2 isolates, albeit to a different degree in
291 each isolate (**Figure 6A** and **Table S3).** In total, there were 1,821 minor variants, distributed
292 across all genomic regions (**Figure S5; Table S3**). For both SNPs and INDELs, intergenic minor
293 variants outnumbered those in genes (genic), likely reflecting the higher selective pressures
294 against unfavorable mutations in coding regions. The neonatal isolate DISS29 had 8-10-fold
295 higher levels of minor variants than other neonatal isolates (**Figures 6A and S5**), and these
296 variants were often present at a higher frequency or penetrance of the minor allele than observed
297 in other neonatal isolates (**Figures 6B and S5).** We further examined the distribution of minor
298 variants that occurred in genes, and found that nearly every HSV-2 protein harbored minor
299 variants in at least one neonatal isolate (**Figure 6C**). Only UL3, UL11, UL35, and UL55 were
300 completely devoid of minor variants. Three of these genes (UL3, UL11, UL35) were also devoid
301 of AA variations at the consensus level (**Figure 5).** These data revealed the breadth of potential
302 contributions of minor variants to neonatal HSV-2 biology, which could undergo selection over

303 time or in specific niches.

304 **Coding variations identified in neonatal HSV-2 isolates associated with CNS disease**

305 To understand how viral genetic variants might relate to clinical disease, we assessed whether
306 any of the consensus level coding variations identified in our group of ten neonatal HSV samples
307 segregated with clinical disease features. A number of AA variations were shared by the CSF-
308 derived isolates CNS11 and DISS14, which formed the largest plaques *in vitro*. These included
309 AA variants at the level of the consensus genome in the HSV-2 genes encoding glycoprotein K
310 (gK, UL53 gene: V323M), glycoprotein I (gI, US7 gene: R159L and P215S), UL8 (R221S), US2
311 (F137L), and glycoprotein G (gG, US4 gene: R338L, S442P, and E574D) (**Figure 7**). The gI
312 variants exist individually in other viral isolates obtained from neonates with CNS disease
313 (**Table S4**). Isolates collected from infants experiencing disseminated disease with CNS
314 involvement also shared a variant in the HSV-2 UL20 gene (P129L) (**Figure 7**). One variant in
315 the HSV-2 UL24 gene (V93A) was shared only by isolates from infants with SEM disease, with
316 all isolates from infants with CNS disease containing a valine at this position **(Figure 7).** The
317 sample size of these comparisons was constrained by the overall limits of neonatal HSV-2 isolate
318 availability, and is too small to evaluate statistical significance for any of these associations.
319 While CNS11 and DISS14 shared several variants listed in **Figure 7**, these viral genomes were
320 not similar at the consensus genome level (**Figure 4**). These isolates also had non-shared coding
321 differences in the same genes that contained shared variants (e.g. gK, UL8, gG). This indicates a
322 potential for convergent evolution at these loci. Many of the coding variations identified in this
323 dataset involve viral proteins that are known to modulate cell-to-cell spread (68–72) and/or
324 contribute to neurovirulence in mouse models of CNS infection (70, 73–79) (**Figure 7** and **Table**

325 **S4**), however, their role in human disease has not yet been assessed.

326 Discussion

327 Host factors have not been identified to explain the >50% of neonates experiencing invasive
328 CNS or disseminated forms of HSV infection. There is growing evidence that most
329 herpesviruses, including HSV-1 and HSV-2, contain significant genetic variation (27, 53, 56,
330 80–82). The potential contributions of viral genetic variation to clinical disease in neonates
331 therefore warrant exploration. Here, we analyzed genetic and phenotypic diversity for HSV-2
332 isolated from ten neonatal patients spanning two clinical studies (8, 9, 61). Although this group is
333 small, the associations identified here provide the first insights into the potential impact of viral
334 variability on clinical outcomes in neonatal HSV disease, and serve as a starting point for further
335 mechanistic investigation. We found that these ten neonatal HSV-2 isolates exhibited diverse
336 growth characteristics in culture, with larger plaque-forming isolates observed more often in
337 infants with CNS as opposed to SEM disease. Furthermore, we established that enhanced viral
338 spread through culture was the main contributor to this large plaque formation. Using
339 comprehensive comparative genomics, we further demonstrated that these neonatal HSV-2
340 isolates contained extensive genetic diversity both within and between hosts. These data revealed
341 several specific viral genetic variations that were associated with cases of CNS disease, in
342 proteins known to contribute to cell-to-cell spread and/or neurovirulence in mouse models of
343 CNS disease. Further studies are required to determine the impact of these variations on HSV-2
344 neurovirulence and progression to CNS disease.

345 Genomic comparison of these neonatal isolates revealed a wide range of genetic diversity. At the

346 consensus genome level, which reflects the most common allele in each viral population, we
347 found that coding differences between strains were as numerous as between previously described
348 sets of adult HSV-2 isolates **(Figure 5)** (80–82). Furthermore, the specific genetic variations
349 associated with neonatal CNS disease in our study can also be found in genital HSV-2 isolates
350 which are not associated with CNS disease in adults. This suggests that the dramatic differences
351 in clinical manifestations following HSV-2 infection in neonates, with significantly higher rates
352 of invasive CNS infection, are not due to unique neonatal HSV-2 strains. However we did detect
353 several outliers in the comparison between the rate of non-synonymous to synonymous
354 nucleotide differences (the dN/dS ratio) of several genes in neonatal HSV-2 isolates versus those
355 previously sequenced from adult patients (**Figure S4**). The genes encoding the putative virulence
356 factor US8A (83, 84) and the capsid triplex protein VP19C (UL38) (85, 86) have a markedly
357 higher dN/dS ratio in neonates, while that of glycoprotein C (gC; UL44) is notably lower in
358 neonates than in adults (**Figure S4**). These differences in dN/dS ratio may reflect the distinct
359 host environment of these isolates. For example viruses isolated from adult patients, often with
360 recurrent genital infection, may have increased diversity of surface proteins such as gC due to
361 selection for viral immune evasion (87, 88). The lower variability in gC (UL44) in neonates
362 could result from their immunologically naïve state, and/or the shorter duration of neonatal
363 infection prior to virus isolation (Table 1). The genes observed to have higher dN/dS ratios in
364 neonatal isolates than in adults could result either from a loosening of selective pressures (i.e.
365 drift) or from driving forces unique to the neonatal environment that remain to be understood.
366 The comparison of additional isolates and ongoing characterization of under-studied proteins
367 such as US8A (84) will help to distinguish these possibilities.

368 At the level of specific genetic variations in individual proteins, we detected a few fully

369 penetrant patterns that distinguish one clinical group from another. Figure 7 highlights all of the
370 fully-penetrant, group-specific variations that we detected in the two CSF-derived strains, two
371 disseminated strains, and three SEM strains available for study. However other loci of potential
372 interest exist if we consider those genetic variants found in a majority, but not all, of the
373 neuroinvasive (CNS and DISS) strains – e.g. variations in the envelope glycoprotein gH (UL22),
374 the viral serine/threonine kinase US3, the viral thymidine kinase (UL23), the major capsid
375 protein UL19, and the DNA polymerase processivity factor UL42. The characterization of
376 additional neonatal isolates will no doubt improve the clarity of these comparisons, and help to
377 distinguish consistent patterns from those detected by chance due to the clinical limitations of the
378 infant sample pool. Regardless of the specific viral genetic candidates under consideration, we
379 hypothesize that viral variations impacting neonatal outcomes could either act by conferring
380 enhanced neurovirulence, or alternatively, that they could act by limiting the rate of viral spread
381 or degree of neuroinvasion. For instance, it is tempting to speculate that variations in
382 glycoprotein I (US7) might be associated with enhanced viral spread, due to their observation in
383 several large-plaque-associated viruses in this study, and prior data demonstrating the
384 involvement of gI in both immune evasion and neuronal spread (see **Table S4**) (69, 89, 90).
385 Conversely, the UL24 V93A variant, which was only observed in SEM-derived isolates and is
386 relatively rare among adult HSV-2 isolates as well, could be a potential example of a spread-
387 limiting variation. UL24 function is required to disperse nucleolin during lytic HSV-1 infection
388 (91, 92), and mutation of UL24 has been associated with a loss of neuroinvasion in animal
389 models (39–41). Further research will be needed to build stronger genetic associations with
390 additional neonatal isolates, and to expand upon prior studies by testing these specific genetic
391 variations in animal models. The application of animal models of neonatal infection will also

392 enable the exploration of how non-genetic environmental factors such as dose and timing of viral
393 exposure contribute to the severity and progression of disease, and how viral genetic variations
394 and environmental factors intersect with the host genetic background.

395 At the level of minor variants (MV), which represent rare alleles that exist within each intra-host
396 viral population, we found that the DISS29 harbored 8-10 fold and CNS15 harbored 3-4 fold
397 more MV than other neonatal virus genomes (**Figures 6 and S4**). This could be indicative of a
398 mixed viral population (e.g. a multi-strain infection), decreased polymerase fidelity, or the
399 presence or absence of host selective pressure (28, 27). Diversity in viral populations has also
400 been observed in congenital HCMV infection (30–35). However, this is the first time that
401 evidence has been found for this level of viral population diversity with HSV-2. These minor
402 genotypes may be selected or genetically isolated in particular niches (e.g. CSF), as observed in a
403 comparison of VZV skin vesicles (26), or by antiviral drug selection, as was recently
404 demonstrated in two adults with genital HSV-2 infection (93). All of the isolates sequenced in
405 this study were collected at the initial time of diagnosis (≤19 days old), prior to acyclovir
406 treatment and prior to the development of an HSV-specific immune response. It would therefore
407 be compelling to examine the viral genome population from serial patient isolates over time to
408 identify shifts in the frequency of MV due to antiviral or immune selection. Isolates from
409 different body sites of the same patient could likewise be compared to determine whether
410 particular genotypes are enriched in different body niches.

411 This comparison of viral genotype to clinical phenotype revealed associations between neonatal
412 CNS disease and several viral protein variants that may impact neurovirulence through
413 modulation of cell-to-cell spread. Although the sample set in this proof of concept study is small,

414 we observed potential patterns that warrant exploration in a larger dataset. It is important to
415 acknowledge that there is limited availability of samples from neonatal infection, both due to the
416 rarity of these infections, and the fragility and limited body size of the infected infants. These
417 natural circumstances lead to minimal sample collection from infected neonates. The finding that
418 CNS-associated isolates in our study often exhibit enhanced spread between cells in culture,
419 particularly those derived directly from the CSF, suggests that one or more of these variants
420 could be functionally significant. Coding differences in viral proteins not known to contribute to
421 neurovirulence were also found to be associated with neonatal CNS disease, and represent
422 potential novel contributions to invasive infection. These promising results warrant exploration
423 in a larger study, ideally with isolates from multiple time points and/or body sites from each
424 infected infant. This would enable a better understanding of how overall viral genetic diversity
425 contributes to neuroinvasion.

426 # Methods

427 ## Viruses

428 Viruses were collected from neonates enrolled in clinical studies (8, 9, 61) by the Collaborative
429 Antiviral Study Group (CASG) at the University of Alabama Birmingham (UAB). Samples were
430 collected from either the cerebrospinal fluid (CSF) or skin. Enrollment in original studies was
431 evenly split between males and females, and included both black and white patients. Clinical
432 morbidity score was determined at 12 months of life as previously defined (94, 12). Initial
433 collection of samples, use of samples in this study, and use of de-identified clinical information
434 was approved by the UAB Institutional Review Board. See Supplemental Information for

435 additional details of this and subsequent methods.

436 **Cell culture**

437 Human lung fibroblast MRC-5 cells (ATCC®, CCL-171), African green monkey kidney Vero
438 cells (ATCC®, CCL-81), and human epithelial bone osteosarcoma U2OS cells (ATCC®, HTB-
439 96) were cultured under standard conditions. Cell lines were authenticated by ATCC prior to
440 purchase, and were confirmed to be mycoplasma free throughout experiments by periodic testing
441 (LookOut Mycoplasma, Sigma).

442 **Virus culture**

443 Viruses were cultured at the time of diagnosis and snap frozen after 1 passage. Each viral isolate
444 was then passaged 3 times on MRC-5 cells at a MOI of 0.01, with harvest at the time of
445 complete cytopathic effect (between 50-70 h). Viral stocks were titered on either Vero cells (100
446 h) or U2OS cells (48 h) under a methylcellulose overlay. Plaque size and morphology did not
447 change for any viral isolates over the course of virus stock expansion.

448 **Plaque measurements**

449 Plaques were stained with 0.5% methylene blue. Serial 4X brightfield images were collected on
450 an EVOS FL Auto Imaging System and stitched by EVOS software (University of Pennsylvania
451 Cell and Developmental Biology Microscopy Core). Plaque area was measured using ImageJ
452 software.

453 **Genome copy number estimation by quantitative PCR for UL27**

454 DNA was extracted and quantitative PCR was performed with primers specific to the viral
455 glycoprotein B gene (gB; UL27) (43). Absolute quantification was calculated based on a
456 standard curve of HSV-1 strain F nucleocapsid DNA (59).

457 **Viral entry assay**

458 Monolayers of Vero cells were cooled to 4°C for 30 minutes prior to infection with 100 PFU of
459 each viral isolate. After 1 h of viral incubation at 4°C, unbound virus was removed by washing
460 and cells were moved to 37°C. At 0, 10, 20, 30, 45, or 60 minutes, a low-pH citrate buffer was
461 applied to inactivate extracellular virus. For each condition, parallel infections were performed
462 without the addition of citrate solution (control). Cell monolayers were washed and allowed to
463 form plaques under methylcellulose for 100 h. Viral entry was quantified as the fraction of
464 plaques formed following citrate buffer application, where 100% is the number of plaques
465 formed on a monolayer not treated with citrate buffer (control).

466 **Single-step and multi-step growth curves**

467 Growth curves of HSV infection were performed in Vero cells. At 2 hpi, 0.1% human serum was
468 added to reduce cell-free spread of virus. Single-step growth curves were performed at MOI=5,
469 and multi-step growth curves at MOI=0.001, as defined by titering viral stocks on U2OS cells.
470 Every 24 h, the supernatant was removed and media containing 0.1% human serum reapplied.

471 **Immunocytochemistry**

472 Immunocytochemistry was performed as previously described (95). Infection was detected with
473 rabbit anti-HSV primary antibodies (Agilent Dako, B0114) and fluorophore-conjugated anti-
474 rabbit secondary antibodies (Invitrogen, A-11008). Cell nuclei were counterstained with DAPI.
475 5X images were collected with a Leica DM6000 wide field microscope equipped with a
476 Photometrics HQ2 high resolution monochrome CCD camera, and processed with LAS AF
477 software (UPenn Cell and Developmental Microscopy Core). 10X images were collected on an
478 EVOS FL Auto Imaging System and stitched using EVOS software (UPenn Cell and
479 Developmental Microscopy Core). Exposure and gain were optimized within each experiment
480 for one virus at the 72 h time point and applied identically to each image within that experiment.
481 Subsequent image processing (ImageJ) was applied equally to all images in a given experiment.

482 **Immunoblotting**

483 Immunoblotting was performed as previously described (95). Equal amounts of whole cell lysate
484 were separated by SDS-PAGE. Membranes were immunoblotted with antibodies raised against
485 total HSV (Agilent Dako, B0114); glycoproteins (g)C, gD, gE, gH, and VP5 (all gifts from Gary
486 Cohen); ICP8 (gift from David Knipe); and GAPDH (GeneTex, GTX100118).

487 **Viral DNA isolation and Illumina sequencing**

488 Viral nucleocapsid DNA for genome sequencing was prepared by infecting MRC-5 cells at an
489 MOI ≥5 as previously described (96, 97). Viral nucleocapsid gDNA was sheared using a Covaris
490 M220 sonicator/disruptor under the following conditions: 60s duration, peak power 50, 10% duty
491 cycle, at 4°C. Barcoded sequencing libraries were prepared using the Illumina TruSeq low-

492 throughput protocol according to manufacturer's specifications and as previously described (60,
493 98). The quality of sequencing libraries was evaluated by Qubit (Invitrogen, CA), Bioanalyzer
494 (Agilent), and qPCR (KAPA Biosystems). Paired-end sequencing (2 × 300bp length) was
495 performed on an Illumina MiSeq, according to manufacturer's recommendations (17pM input).

496 ***De novo* genome assembly**

497 A consensus genome was assembled for each viral isolate using a previously described Viral
498 Genome Assembly (VirGA) bioinformatics workflow (60). Annotation of new genome
499 sequences was guided by the HSV-2 reference genome (strain HG52; GenBank NC_001798)
500 based on sequence homology (99).

501 **Comparative genomics and phylogenetic analysis**

502 The 10 neonatal HSV-2 genomes were aligned with all annotated HSV-2 genomes available in
503 GenBank (see **Table S1** for full list of 58 genomes; all derived from adults) using MAFFT (100).
504 The genome-wide alignment used a trimmed genome format (lacking the terminal repeats) to
505 avoid giving undue weight to these duplicated sequences. The MAFFT alignment was used to
506 generate a NeighborNet phylogenetic network in SplitsTree with Uncorrected P distances (49,
507 101, 102), as well as a Neighbor-Joining tree (Jukes-Cantor, 1000 bootstraps) in MEGA Omega
508 for Windows (103). A diverse subset of ten adult HSV-2 isolates was selected for protein-level
509 comparisons with the ten neonatal isolates (indicated in **Table S1** with an asterisk). ClustalW2
510 was used to construct pairwise nucleotide alignments between whole genomes, and pairwise
511 amino acid alignments for each gene and protein (104). Pan-HSV2 comparisons excluded three
512 viral proteins for which sequences are not fully determined in most published strains, likely due

513 to the high G+C-content and numerous tandem repeats in these regions: ICP34.5 (RL1 –
514 annotated/complete in only 9 genomes from **Table S1**), ICP0 (RL2 – annotated/complete in only
515 8 genomes from **Table S1**), and ICP4 (RS1 – annotated/complete in only 6 genomes from **Table**
516 **S1**) (80–82). Custom Python scripts were used on these alignments to identify nucleotide and
517 AA differences between samples.

518 **Minor variant detection & quantification**

519 Minor variants (MV) were detected using VarScan v2.2.11 (mpileup2snp and mpileup2indel
520 commands) (105), using the following parameters to differentiate true MV from technical
521 artifacts (26): minimum allele frequency ≥ 0.02 (2%); base call quality ≥ 20; read depth ≥ 100;
522 independent reads supporting minor allele ≥5. MV with directional strand bias ≥ 90% were
523 excluded. The genomic location and potential impact of each MV was assessed using SnpEff and
524 SnpSift (106, 107). We use the conservative cutoff of 2% for detection of minor variants both to
525 avoid false positive signals and to provide data that is comparable to recent examples in the
526 genomic analysis of herpesviruses in clinical samples (108, 28, 109).

527

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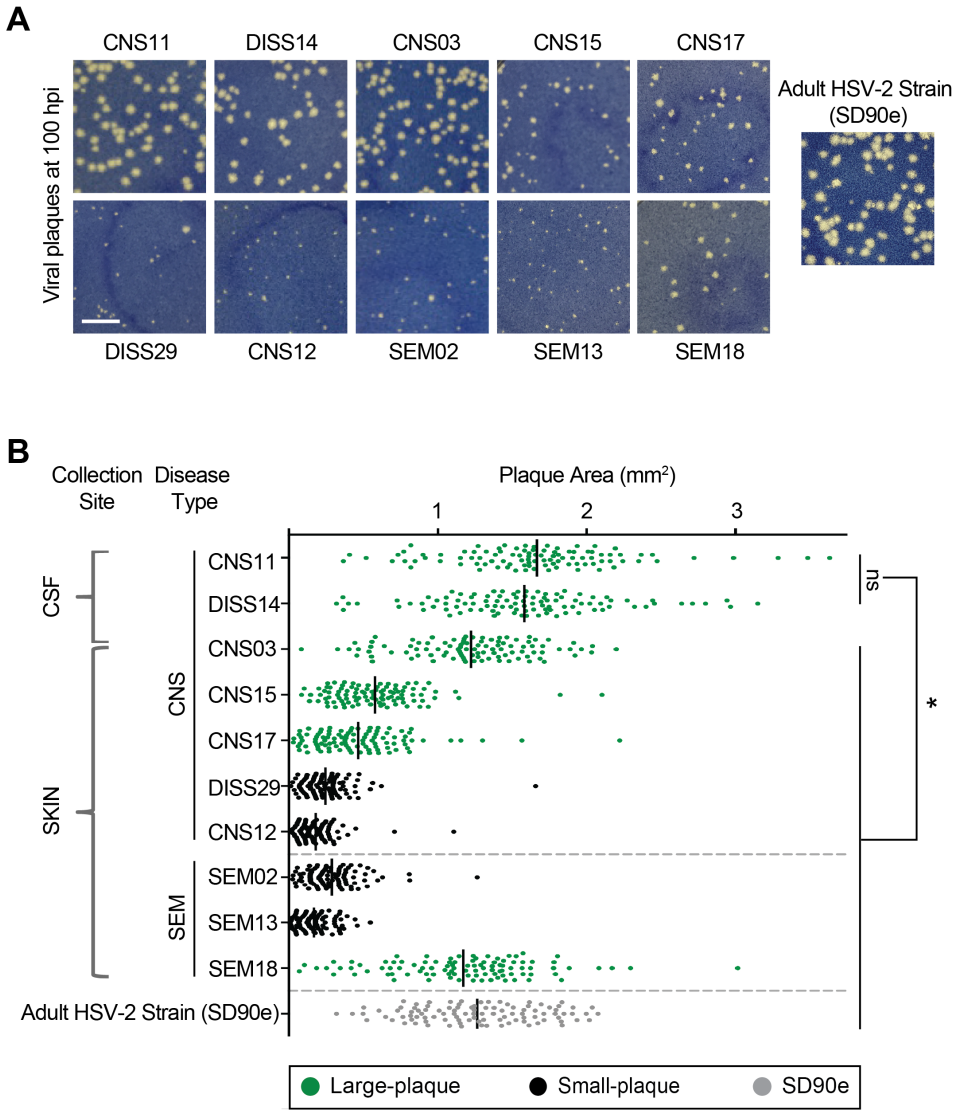
541 Author Contributions

542 Writing – Original Draft: LNA, CB, AND, and MLS; Writing – Review & Editing: LNA, MDW,
543 and MLS; Conceptualization: LNA and MLS; Investigation: LNA, CB, AND, DWR, UP;
544 Resources: DWK, MNP, and RJW; Funding Acquisition: MLS, RJW, and MDW.

545 Declaration of Interests

546 The authors declare no competing interests.

547 # Figure Legends



548 **Figure 1.**

549 **Neonatal HSV-2 isolates generate different sized plaques in culture.**

550 (**A**) Representative plaques are shown after virus incubation on Vero cells for 100 h. The
551 previously described low-passage adult HSV-2 strain SD90e is shown for comparison (64). Scale
552 bar = 5mm. (**B**) Quantification of plaque area on Vero cells. Dots represent 100 individually
553 measured plaques and black bar represents the mean. Each green isolate (large-plaque) is
554 statistically larger than each black isolate (small-plaque). Black isolates are not statistically

555 different from one another. Additionally, each CSF-derived isolate is statistically larger than all
556 other isolates shown. For all statistics, $p<0.05$ by one-way ANOVA followed by Holm-Sidak's
557 multiple comparisons test.

558

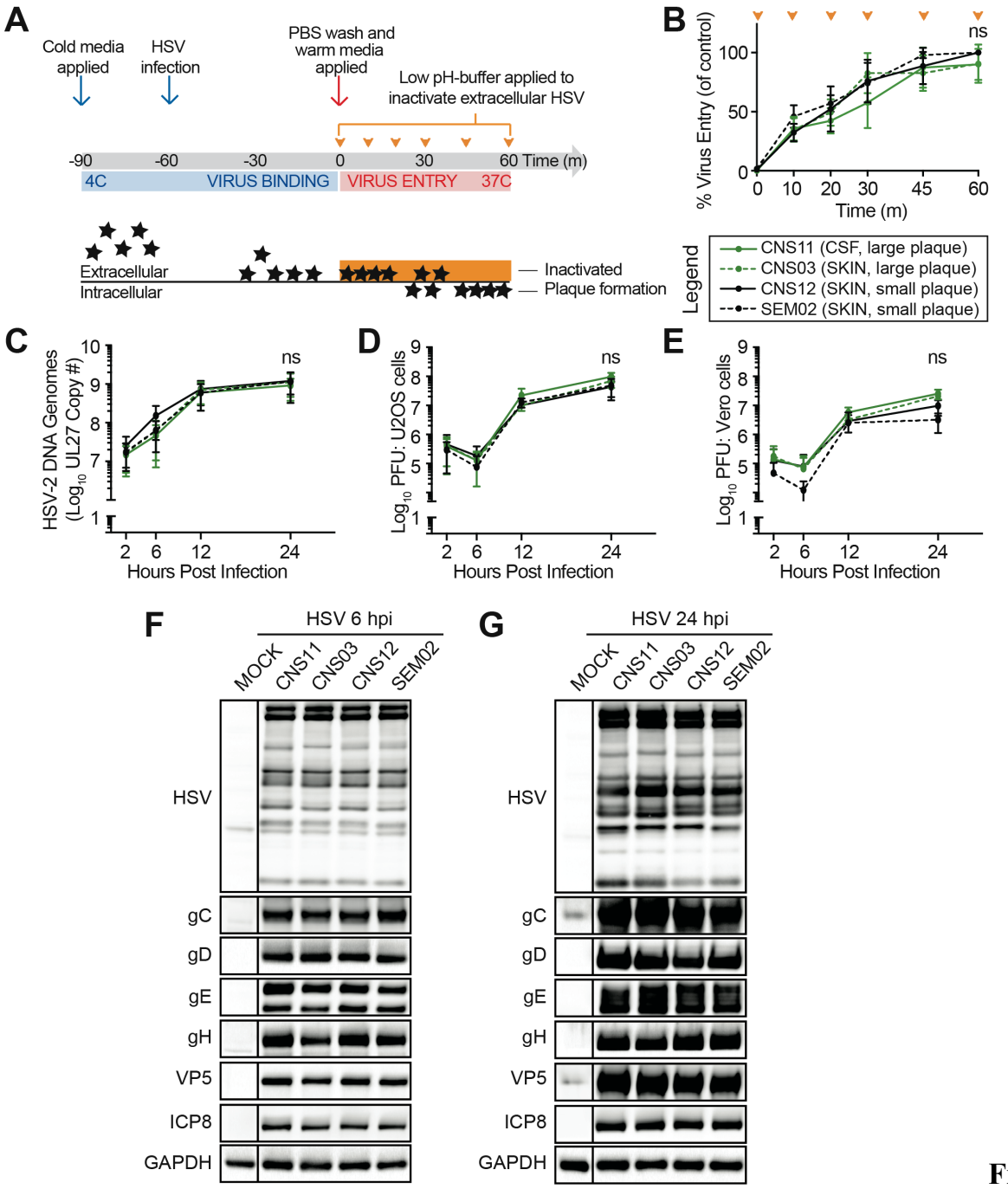
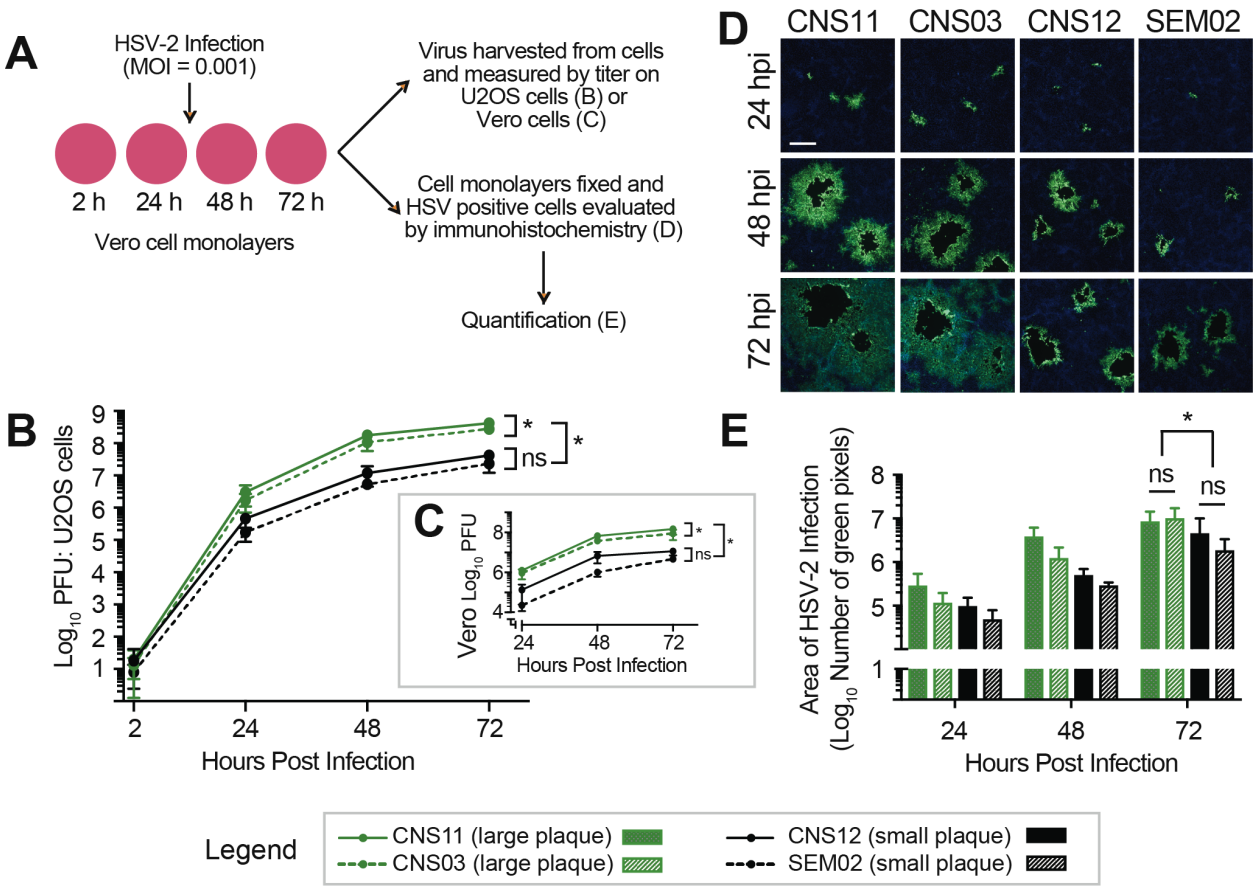


Figure 2. Increased plaque size in culture is not determined by viral entry, DNA replication, protein expression, or infectious virus production.

Viral growth characteristics were compared for representative neonatal isolates, including large-plaque-formers (green) and small-plaque-formers (black). **(A-B)** Viral entry kinetics. **(A)** Viral

564 isolates were applied to Vero cell monolayers at 4°C for 1 h to allow virus binding, then moved
565 to 37°C to allow virus entry. Extracellular virus was inactivated by a low-pH buffer at the times
566 indicated (orange arrowheads). Cell monolayers were washed and overlaid with methylcellulose.
567 Plaques were scored after 100 h of incubation. **(B)** Viral entry was quantified as the fraction of
568 plaques formed following citrate buffer application, where 100% is the number of plaques
569 formed on a monolayer not treated with citrate buffer (control). These data represent three
570 independent experiments. Two-way ANOVA followed by Tukey's multiple comparison test was
571 applied. **(C-E)** Single-cycle viral replication kinetics. Vero cell monolayers were infected at
572 MOI=5 and incubated in the presence of 0.1% human serum. Cell monolayers were harvested at
573 the time points indicated. **(C)** The quantity of viral genomes present was evaluated by qPCR for
574 UL27. Infectious virion production (titer) was evaluated by plaque formation on U2OS **(D)** or
575 Vero **(E)** cells. These data represent three independent experiments. Two-way ANOVA followed
576 by Tukey's multiple comparison test was applied. **(F-G)** Protein production. Vero cell
577 monolayers were infected at MOI=5 for 6 h **(F)** or 24 h **(G)**. Whole cell lysates were subjected to
578 immunoblot analysis with the following antibodies: gC/gD/gE/gH, four virion glycoproteins;
579 VP5 (UL19), capsid protein; ICP8 (UL29), viral single-strand DNA-binding protein; HSV, viral
580 antibody against whole HSV-1; and GAPDH, cellular glyceraldehyde-3 phosphate
581 dehydrogenase as a loading control.

582



583

584 **Figure 3. Enhanced viral cell-to-cell spread contributes to increased plaque size in**

585 **culture.**

586 (**A**) The rate of viral spread in Vero cells was compared for representative neonatal isolates,

587 including large-plaque-formers (green) and small-plaque-formers (black). Vero cell monolayers

588 were infected at MOI=0.001 in the presence of 0.1% human serum, which was replenished every

589 24 h. Samples were harvested at each time point and viral titer was evaluated by plaque

590 formation on U2OS cells **(B)** or Vero cells **(C).** These data represent three independent

591 experiments. Two-way ANOVA followed by Tukey's multiple comparison test, *p<0.0001 at 72

592 h. **(D)** In parallel experiments, HSV positive cells (green) were evaluated by

593 immunofluorescence. Cell nuclei are counterstained with DAPI (blue). Scale bar = 200 μm.

594 Images are representative of three independent experiments. Images of the entire 10 mm

595 coverslips were then captured and stitched to create a composite image (see **Figure S2**). (**E**) The
596 total number of immunofluorescent (green) pixels was quantified for each coverslip. Two-way
597 ANOVA followed by Tukey's multiple comparison test, *$p<0.05$ at 72 h.

598

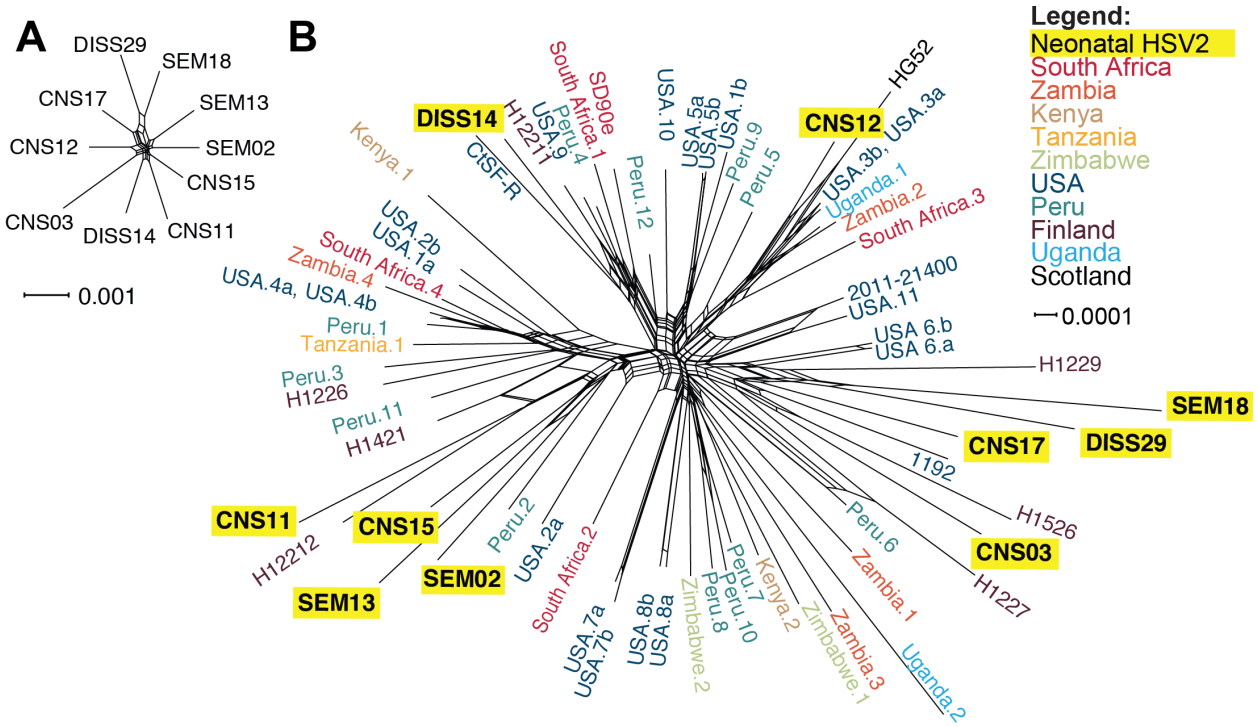


Figure 4. Neonatal HSV-2 genomes are genetically distinct from one another and encompass a broad range of known HSV-2 genetic diversity.

A phylogenetic network constructed among neonatal HSV-2 genomes (**A**), or neonatal and adult HSV-2 genomes (**B**), reveals the wide genetic distribution of these unrelated isolates. HSV-2 genomes have been previously noted to lack geographic separation into clades (41, 42, 82). The network was created using SplitsTree4, from a MAFFT trimmed genome alignment. See **Figure S3** for a comparison tree constructed using a neighbor-joining (NJ) algorithm. See **Table S1** for a complete list of accessions, geographic origins, and references for all 58 adult HSV-2 strains.

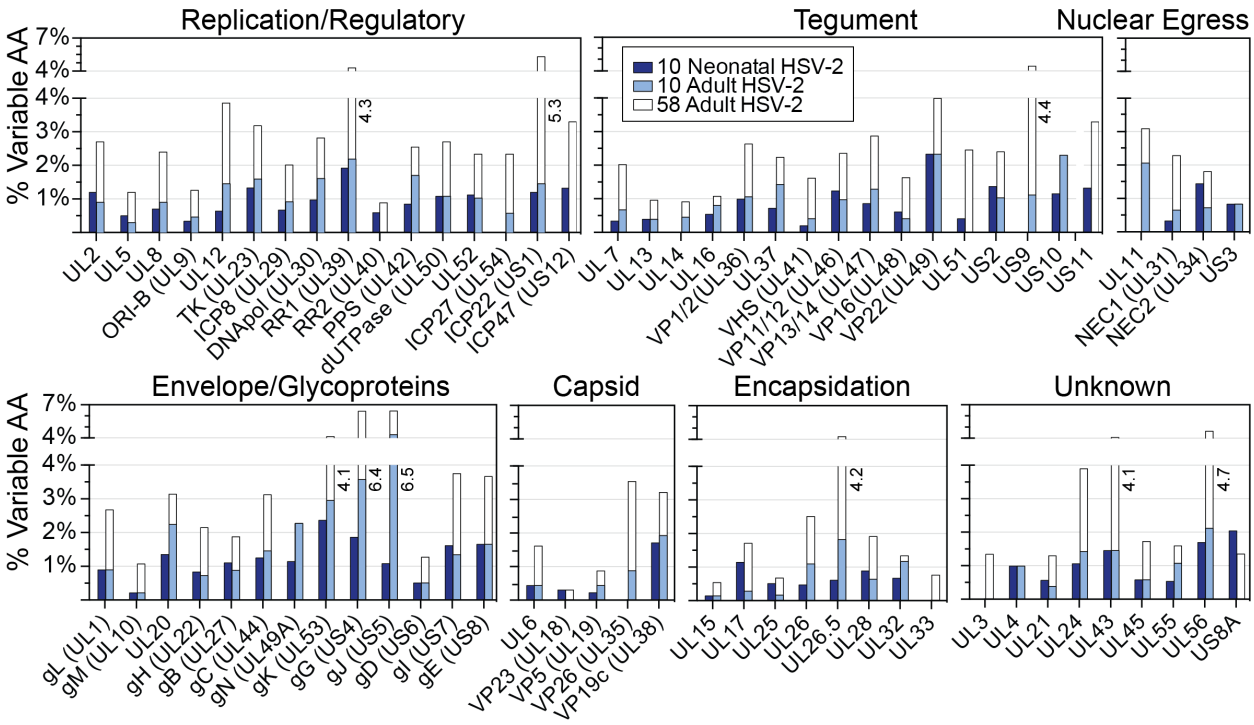


Figure 5. Neonatal HSV-2 proteins contain a similar degree of consensus-level amino acid (AA) differences as adult HSV-2 isolates.

HSV-2 proteins are grouped by function, and the percentage of variable AAs in each protein (#AA differences divided by protein length) are plotted for the 10 neonatal isolates (dark blue), for 10 representative adult HSV-2 isolates (light blue), and for all 58 annotated adult HSV-2 genomes in GenBank (clear outlines behind light blue boxes). All adult HSV-2 genomes used for this comparison are listed in **Table S1**.

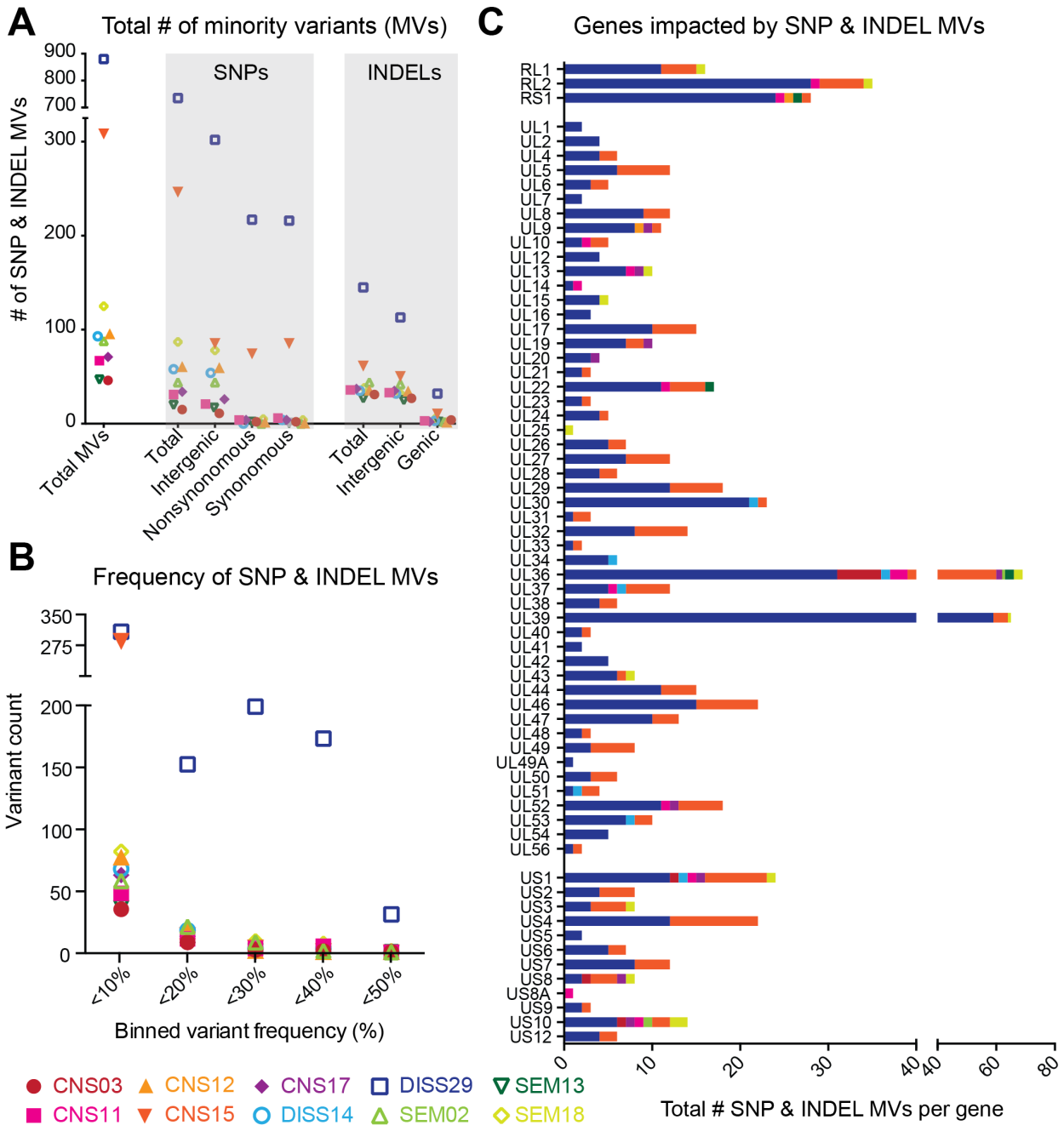
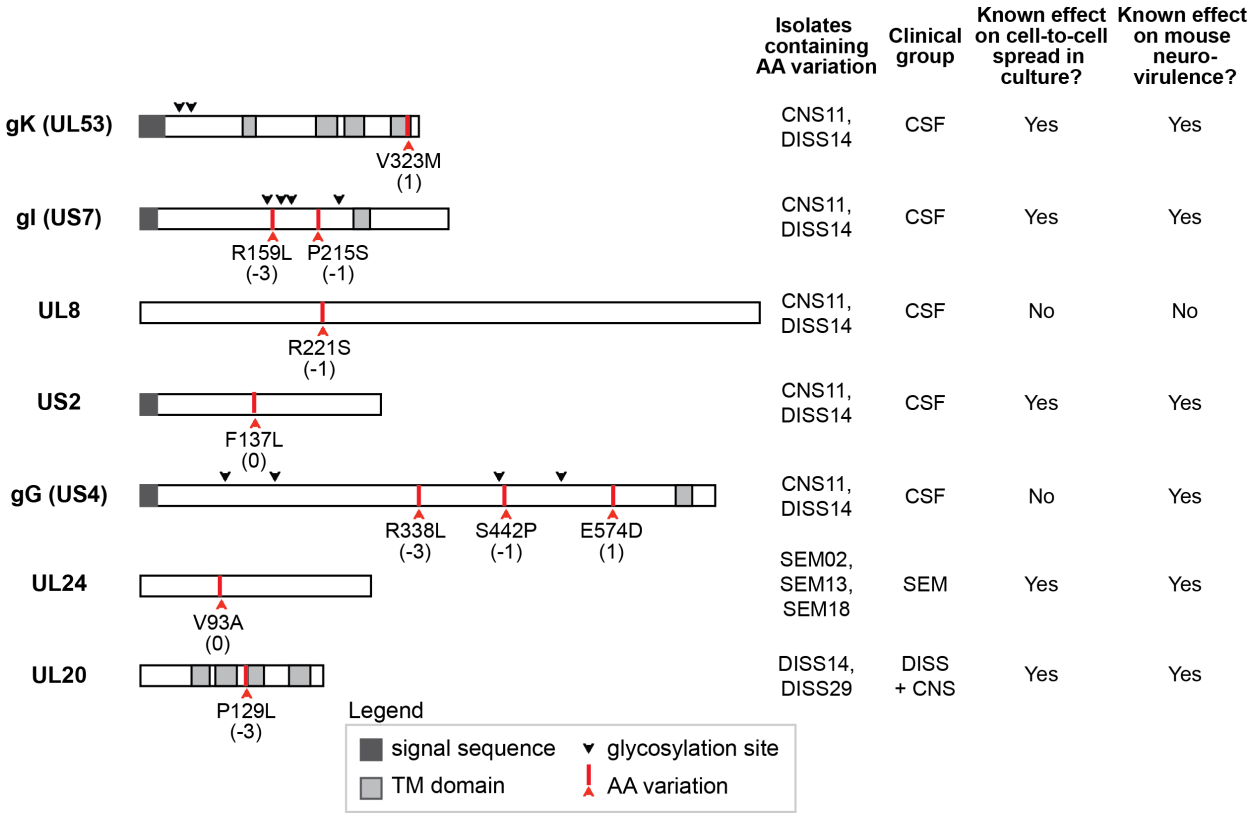


Figure 6. Minor variants expand the range of neonatal HSV-2 coding diversity.

(**A**) Scatter plot indicates the total number of minor variants (MV; y-axis) observed in each neonatal isolate. MV are rare alleles that exist within each viral population, below the level of the consensus genome. The total number of MV on the left is separated into single-nucleotide

623 polymorphism (SNP) vs. insertion/deletion (INDEL) variants on the right (x-axis). The genomic
624 location of each SNP or INDEL variant is also summarized: intergenic vs. inside of genes (genic)
625 for INDELS, and intergenic vs. nonsynonymous or synonymous SNPs inside of genes. (**B**) The
626 frequency, or penetrance, of each minor variant was examined for each isolate. Data (x-axis) was
627 binned in increments of 5% (e.g. 0-<5% frequency, 5-<10% frequency, and so on), and plotted
628 according to the number of MV observed at each frequency (y-axis). SNP and INDEL variants
629 were combined for this analysis. (**C**) Stacked histograms show the number of genic MV (x-axis)
630 located in each HSV-2 coding sequence (gene; y-axis). SNP and INDEL variants were combined
631 for this analysis. UL3, UL11, UL35, and UL55 lacked any minor variants and are not included in
632 the histogram. See **Table S3** for full list of SNP and INDEL MV position and frequency data.

633



634

635 **Figure 7. Several coding variations in neonatal HSV-2 isolates occur in proteins**

636 **known to contribute to cell-to-cell spread and neurovirulence.**

637 The domain structure shown for each HSV protein is based on published literature for both HSV-

638 1 and -2. Red arrows indicate protein-coding variations discussed in the text, with the

639 BLOSUM80 score for each AA substitution listed in parentheses beneath the highlighted

640 difference. More detailed information and references for each protein on the domain structure

641 and regarding cell-to-cell spread and neurovirulence can be found in **Table S4**.

642

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961

962 # Tables

963 **Table 1. Clinical characteristics associated with HSV-2 isolates from ten patients.**

Clinical Isolate*	Clinical Disease at Diagnosis	Sample Source	Morbidity Score - Mental	Morbidity Score - Motor	Patient Age at Disease Onset (days)	Gestational Age at Time of Birth (weeks)	Patient Sex & Race
CNS11	CNS	CSF	4	4	12	37	M, W
DISS14	DISS + CNS	CSF	2	2	7	39	M, W
CNS03	CNS	SKIN	4	4	17	37	M, W
CNS15	CNS	SKIN	3	4	19	36	M, W
CNS17	CNS	SKIN	4	4	17	40	M, W
DISS29	DISS + CNS	SKIN	3	3	5	38	F, B
CNS12	CNS	SKIN	4	4	16	41	F, W
SEM02	SEM	SKIN	1	1	5	38	F, W
SEM13	SEM	SKIN	4	4	11	27	F, B
SEM18	SEM	SKIN	2	2	17	37	F, W

964 * Clinical isolate order based on **Figure 1**.

965

966

967 **Table 2. Genome sequencing statistics for neonatal HSV-2 strains.**

Clinical Isolate*	Average Coverage	Raw Sequence Reads	Used for Assembly**	% viral	Depth > 100	GenBank Accession
CNS11	6,081X	3.5 million	2.8 million	79%	96%	MK105996
DISS14	6,267X	3.8 million	3.0 million	77%	97%	MK106000
CNS03	6,269X	3.9 million	3.1 million	78%	96%	MK105995
CNS15	7,486X	6.1 million	4.4 million	73%	97%	MK105998
CNS17	5,059X	3.1 million	2.3 million	73%	96%	MK105999
DISS29	2,588X	1.4 million	1.1 million	78%	99%	MK106001
CNS12	5,839X	3.9 million	2.8 million	73%	96%	MK105997
SEM02	6,455X	4.8 million	3.7 million	77%	95%	MK106002
SEM13	5,291X	3.4 million	2.4 million	72%	89%	MK106003
SEM18	7,514X	16.9 million	12.2 million	72%	98%	MK106004

968 * Clinical isolate order based on **Figure 1**.
969 ** Numbers reflect the count of sequence read 1 of paired-end reads.
970

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Supplemental Information for

Genotypic and phenotypic diversity within the neonatal HSV-2 population

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Virus source

Viruses analyzed were collected from neonates with HSV-2 infection, enrolled in three published clinical studies (1–3) by the Collaborative Antiviral Study Group (CASG) at the University of Alabama Birmingham (UAB). These HSV isolates originated from patients with SEM, CNS, or disseminated (DISS) disease with CNS involvement (**Table 1**). Samples were collected at the time of diagnosis, prior to initiation of acyclovir therapy. CNS11 and DISS14 were isolated from the cerebrospinal fluid (CSF) and all other viruses were isolated from the skin. CSF was collected from all infants at the time of diagnosis. However, for all of the viruses isolated from the skin of infants with CNS disease (CNS03, CNS12, CNS15, CNS17, and DISS29), the CSF collected at the time of diagnosis was PCR positive but culture negative for HSV. Viral isolates are associated with de-identified clinical information including age, sex, race, and clinical morbidity scores, as approved by the UAB Institutional Review Board (IRB). Enrollment in the original studies was evenly split between males and females, and included both black and white patients. Clinical morbidity score was determined by the CASG after 12 months of life as (1)

normal; (2) mild impairment which includes ocular sequelae (keratoconjunctivitis), speech delay, or mild motor delay; (3) moderate impairment which includes hemiparesis, persistent seizure disorder, or developmental delay of less than or equal to 3 months adjusted developmental age; (4) severe impairment which includes microcephaly, spastic quadriplegia, chorioretinitis or blindness, or a serious developmental delay of least 3 months according to the Denver Developmental Assessment Scale (1, 2). These assessments were performed at each collaborating site. Initial collection of samples was approved by the UAB IRB. Use of these samples in this study was approved by the UAB IRB.

Cell culture

Human lung fibroblast MRC-5 cells (ATCC®, CCL-171) were cultured in minimum essential medium Eagle (MEME, Sigma-Aldrich; M5650) supplemented with 10% fetal bovine serum (FBS, Hyclone; SH30071.03), 2mM L-glutamine (Gibco, A2916801) and 1X penicillin-streptomycin (Gibco; 15140122). African green monkey kidney Vero cells (ATCC®, CCL-81) and human epithelial bone osteosarcoma U2OS cells (ATCC®, HTB-96) were cultured in Dulbecco's modified Eagle's medium with high glucose (DMEM, Hyclone; SH30081.02) supplemented with 10% FBS, 2mM L-glutamine, and 1X penicillin-streptomycin. Cell lines were authenticated by ATCC prior to purchase, and were confirmed to be mycoplasma free throughout experiments by periodic testing (LookOut Mycoplasma, Sigma).

Virus culture

Initial viral isolates (passage 1-2) were obtained from CASG samples stored at the UAB. Each isolate had been cultured at the time of diagnosis, aliquoted after one passage, and snap frozen.

To conduct viral genome sequencing and phenotypic assays, each stock was expanded over three serial passages by infecting monolayers of human MRC-5 cells at a MOI of 0.01. Each infection was allowed to progress to complete cytopathic effect (CPE) before harvest (between 50-70 hours), with increasing cell volume at each passage to produce sufficient virions for subsequent phenotypic and genomic comparisons. Viral stocks were titered on monolayers of either Vero cells or U2OS cells, for 100 or 48 hours respectively, to allow plaques to develop. Plaque formation was facilitated by limiting viral diffusion with a methylcellulose overlay. Plaque size and morphology were monitored carefully and did not change for any viral isolates over the course of virus stock expansion.

Plaque measurements

After appropriate incubation plaques were stained with 0.5% methylene blue and allowed to dry. Serial 4X brightfield images were collected on an EVOS FL Auto Imaging System and stitched by EVOS software to create an image of the entire well (University of Pennsylvania Cell and Developmental Biology Microscopy Core). No processing was performed. The area of 100 plaques was measured for each viral isolate using ImageJ software.

Genome copy number estimation by quantitative PCR for UL27

DNA was extracted using a PureLink genomic DNA mini kit (ThermoFisher Scientific). Viral genome copy number was determined using an established assay based on real-time PCR using primers and dual-fluorescent probe specific to viral glycoprotein B gene (gB; UL27) . Samples were assayed alongside a standard curve of HSV-1 strain F nucleocapsid DNA (3), on a ViiA 7 Real-Time PCR System.

Viral entry assay

Monolayers of Vero cells were cooled to 4°C for 30 minutes prior to infection and 100 PFU of each viral isolate was applied to cell monolayers at 4°C for 1 hour to allow virus binding, after which unbound virus was washed from the cells. Cells were then moved to 37°C to allow virus entry. At 0, 10, 20, 30, 45, or 60 minutes, a low-pH citrate buffer was applied to infected cells to inactivate virus that had not penetrated the cellular membrane. At each time point and for each virus, parallel infections were performed without the addition of citrate solution. These served as controls to determine the maximum number of plaques formed. Cell monolayers were washed and overlaid with methylcellulose. Plaques were scored after 100 hours of incubation. Viral entry was quantified as the fraction of plaques formed following citrate buffer application, where 100% is the number of plaques formed on a monolayer not treated with citrate buffer (control).

Single-step and multi-step growth curves

Virus diluted in basic growth media containing 2% FBS was applied to near confluent monolayers of Vero cells and allowed to adsorb for 1 hour. Virus was removed and cells were incubated at 37C for the duration of infection. At 2 hpi, media containing 0.1% human serum was added to reduce the contribution of cell-free spread of virus. Single-step growth curves were performed at MOI=5 as defined by titering viral stocks on U2OS cells, and monolayers harvested at 2, 6, 12, and 24 hpi. Multi-step growth curves were performed at MOI=0.001 as defined by titering viral stocks on U2OS cells, and monolayers were harvested at 2, 24, 48, and 72 hpi. Every 24 hours, the supernatant was removed and media containing 0.1% human serum reapplied. At the conclusion of each infection, cells were washed two times with PBS and

collected by scraping into an equal volume of media.

Immunocytochemistry

Multi-step growth curves were performed as described above in Vero cell monolayers grown on glass coverslips. To terminate each infection, coverslips were washed with PBS and fixed in 4% paraformaldehyde for 15 minutes at room temperature. Cells were permeabilized with 0.5% Triton-X, blocked in 3% BSA, and incubated with polyclonal rabbit antibodies raised against total HSV (Agilent Dako, B0114). After washing, cells were incubated with fluorophore-conjugated anti-Rabbit secondary antibodies (Invitrogen, A-11008) to mark HSV infected cells, and counterstained with DAPI to mark cell nuclei. Coverslips were initially visualized with a Leica DM6000 wide field microscope (UPenn Cell and Developmental Microscopy Core). The 5X images were collected on a Photometrics HQ2 high resolution monochrome CCD camera, and processed with LAS AF software. The 10X images were collected on a EVOS FL Auto Imaging System (UPenn Cell and Developmental Microscopy Core) and stitched using EVOS software. Exposure and gain were optimized within each experiment for one virus at the 72-hour time point and applied identically to each image within that experiment. Any subsequent image processing (ImageJ) was applied equally to all images in a given experiment.

Immunoblotting

Whole cell lysates were prepared with 1X LDS sample buffer (NuPage) and equal amounts of lysate were separated by SDS-PAGE. Membranes were immunoblotted with polyclonal rabbit antibodies raised against total HSV (Agilent Dako, B0114); glycoproteins (g)C, gD, gE, gH, and VP5 (all gifts from Gary Cohen); ICP8 (gift from David Knipe); and GAPDH (GeneTex,

GTX100118). Proteins were visualized with Pierce ECL Western Blotting Substrate (ThermoFisher Scientific) and detected using a G:Box imaging system (Syngene).

Viral DNA isolation and Illumina sequencing

Viral nucleocapsid DNA for genome sequencing was prepared by infecting MRC-5 cells at an MOI ≥5 as previously described (4, 5). Viral nucleocapsid gDNA was sheared using a Covaris M220 sonicator/disruptor under the following conditions: 60s duration, peak power 50, 10% duty cycle, at 4°C. Barcoded sequencing libraries were prepared using the Illumina TruSeq low-throughput protocol according to manufacturer's specifications and as previously described (6, 7). The quality of sequencing libraries was evaluated by Qubit (Invitrogen, CA), Bioanalyzer (Agilent), and qPCR (KAPA Biosystems). Paired-end sequencing (2 × 300bp length) was performed on an Illumina MiSeq, according to manufacturer's recommendations (17pM input).

De novo genome assembly

A consensus genome was assembled for each viral isolate using a previously described Viral Genome Assembly (VirGA) bioinformatics workflow (6). VirGA begins by quality-filtering the MiSeq sequence reads and removing sequences that match the host (human) genome. Thereafter VirGA uses a combination of SSAKE *de novo* assemblies run with differing parameters, which are then combined into a single draft genome using Celera and GapFiller (8–10). After quality-checking and iterative improvement of the genome assembly, annotations were transferred from the HSV-2 reference genome (strain HG52; GenBank NC_001798) to each new genome based on sequence homology (11).

Comparative genomics and phylogenetic analysis

The genomes of all 10 neonatal HSV-2 isolates were combined with all annotated HSV-2 genomes available in GenBank (see **Table S1** for full list) and aligned using MAFFT (12). All previously published HSV-2 genomes were derived from infected adults. The genome-wide alignment used a trimmed genome format (lacking the terminal repeats) to avoid giving undue weight to these duplicated sequences. The MAFFT alignment was used to generate a NeighborNet phylogenetic network in SplitsTree with Uncorrected P distances (13–15). A diverse subset of ten adult HSV-2 isolates was selected for protein-level comparisons with the ten neonatal isolates; these are indicated in **Table S1** with an asterisk. GenBank genomes that lacked open reading frame (ORF) and protein annotations were excluded from all comparisons (16, 17). ClustalW2 was used to construct pairwise nucleotide alignments between whole genomes and pairwise amino acid alignments for each gene and protein (18). Pan-HSV-2 comparisons excluded three viral proteins for which sequences are not fully determined in most published strains, likely due to the high G+C-content and numerous tandem repeats in these regions: ICP34.5 (RL1 – annotated/complete in only 9 genomes from Table S1) and ICP4 (RS1 – annotated/complete in only 6 genomes from Table S1), ICP0 (RL2 – annotated/complete in only 8 genomes from Table S1) (16, 19, 20). Custom Python scripts were used on these alignments to identify nucleotide and AA differences between samples.

References for Supplemental Methods

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Supplemental Figures & Legends

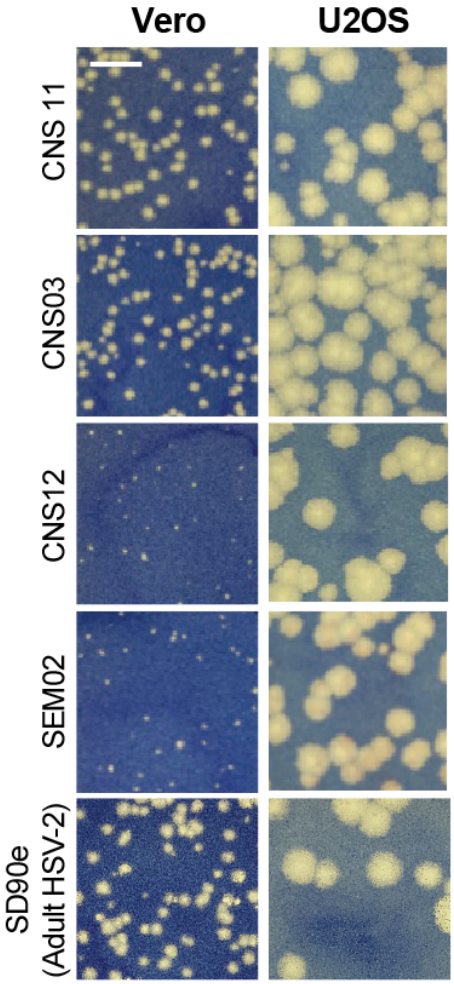

Figure S1. U2OS cells support large plaque formation by all isolates.

Representative plaques are shown after neonatal viruses were allowed to incubate for 100 hours on Vero or U2OS cells. All neonatal isolates were capable of forming large plaques on U2OS cells, which lack innate sensing of viral infection through the STING pathway (21).

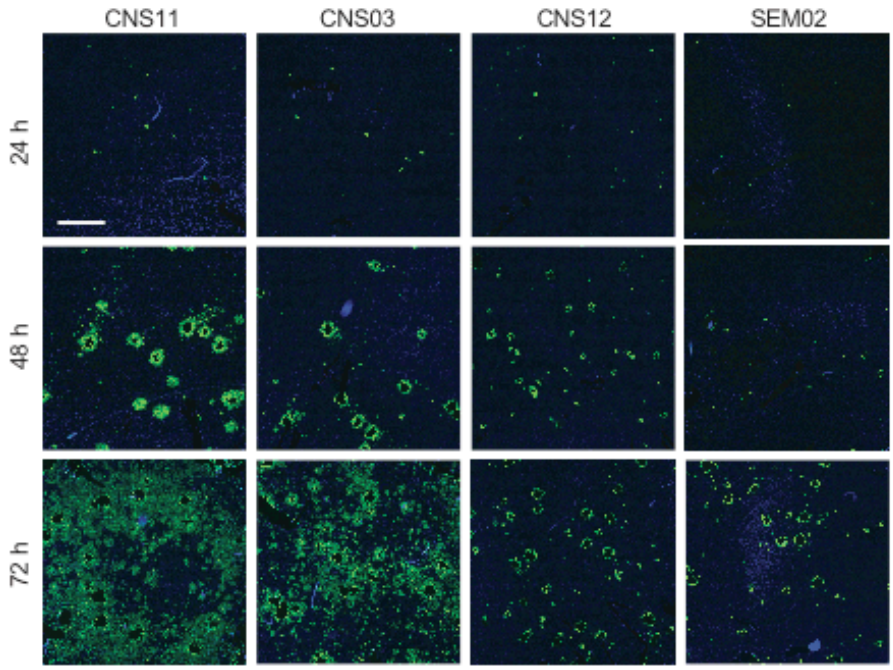

Figure S2. Cell-to-cell spread is enhanced in certain neonatal HSV-2 isolates.

Confluent Vero cell monolayers were infected at MOI=0.001 for the time points indicated, in the presence of 0.1% human serum. HSV positive cells (green) were evaluated at each time point by immunofluorescence. Cell nuclei are counterstained with DAPI (blue). Serial 10X images were obtained on an EVOS FL Auto Imaging System and stitched together to create an image of the entire experimental coverslip. Scale bar = 1mm. These images were quantified in **Figure 3E**.

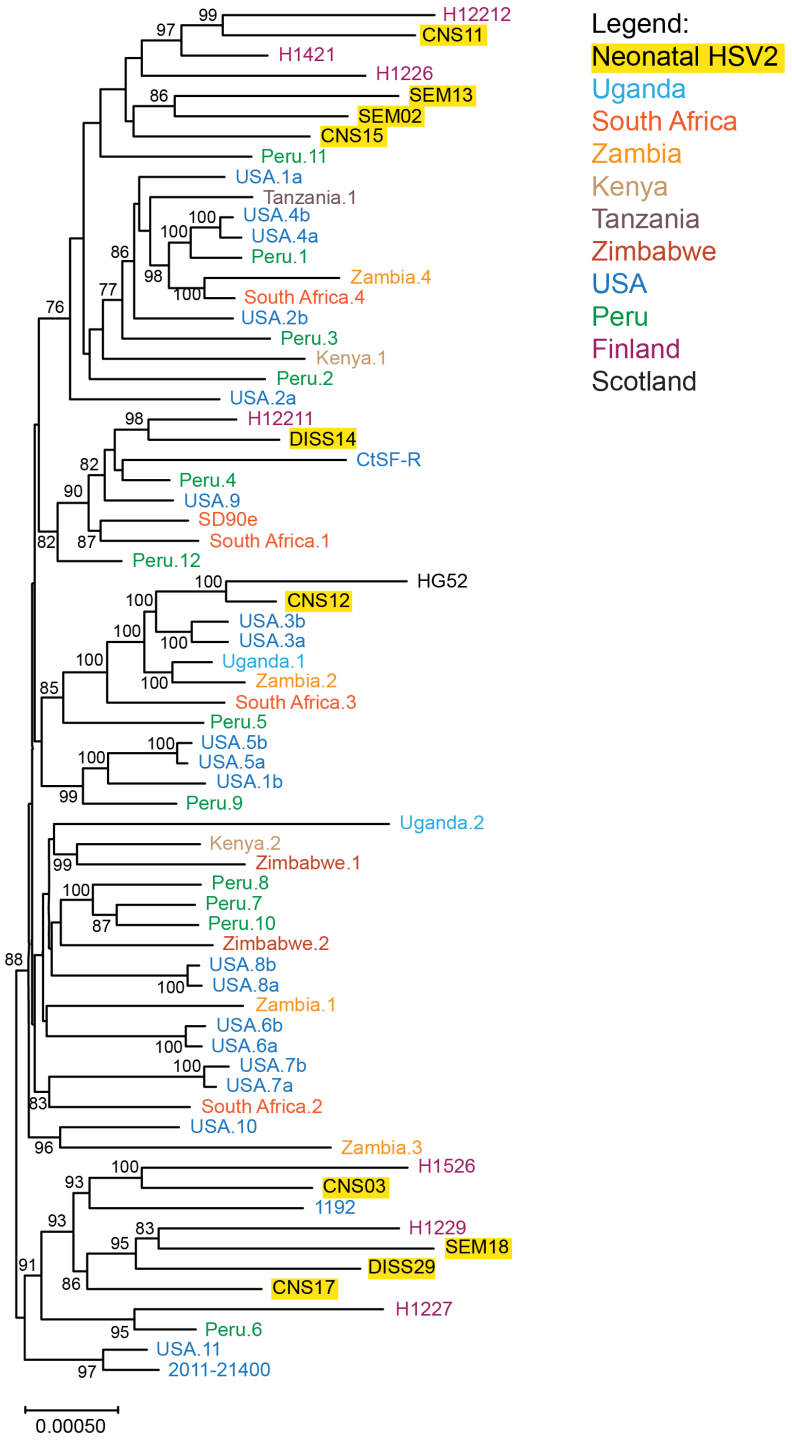

Figure S3. Phylogenetic clustering demonstrates that neonatal HSV-2 genomes are genetically distinct from one another and intermingle with the previously known

range of HSV-2 genetic diversity.

A Neighbor-Joining (NJ) tree network constructed among ten neonatal and 58 adult HSV-2 genomes, revealed the wide genetic distribution of the neonatal isolates. The NJ tree (Jukes-Cantor, 1000 bootstraps) was created in MEGA from a MAFFT trimmed genome alignment. Bootstrap values $\geq$ 70 are shown here. See **Figure 4** for a network graph comparison to this tree. **Table S1** contains a complete list of accessions, geographic origins, and references for all adult HSV-2 strains.

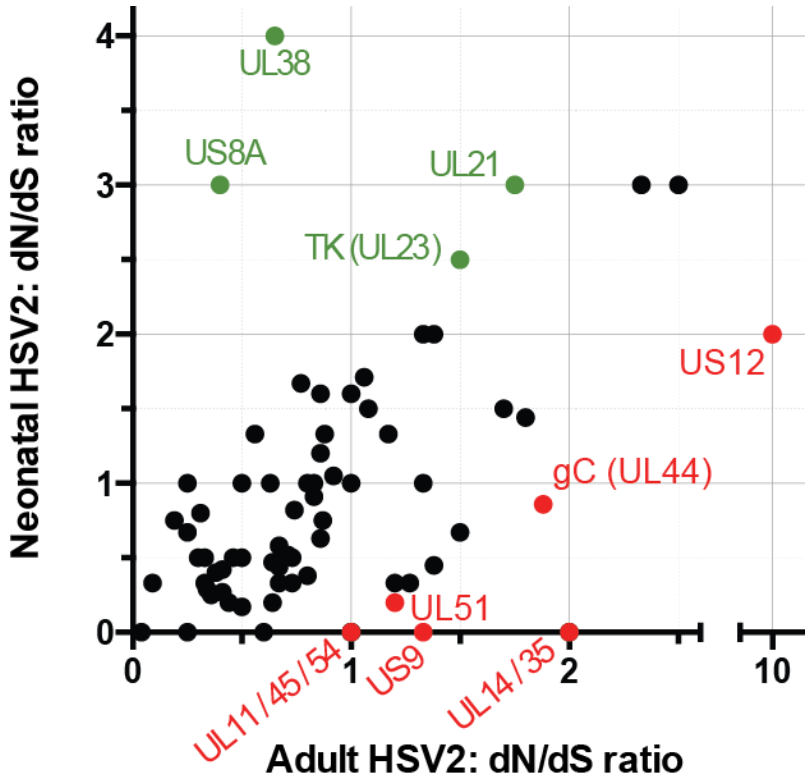

Figure S4. Ratio of non-synonymous to synonymous coding variations in neonatal HSV-2 versus adult HSV-2 strains.

The ratio of non-synonymous (dN) to synonymous (dS) coding variations were plotted for each HSV-2 protein. The x-axis value represents the average dN/dS ratio for each protein in 58 adult HSV-2 strains, while the y-axis value represents the average dN/dS ratio in 10 neonatal isolates. Proteins with a difference in average dN/dS ratios ≥1 in neonatal vs. adult HSV-2 genomes are labeled (green indicates a higher average dN/dS in neonatal HSV-2 genomes; red indicates a higher dN/dS in adult HSV-2 genomes). The average dN/dS ratios for all proteins are listed in **Table S2**.

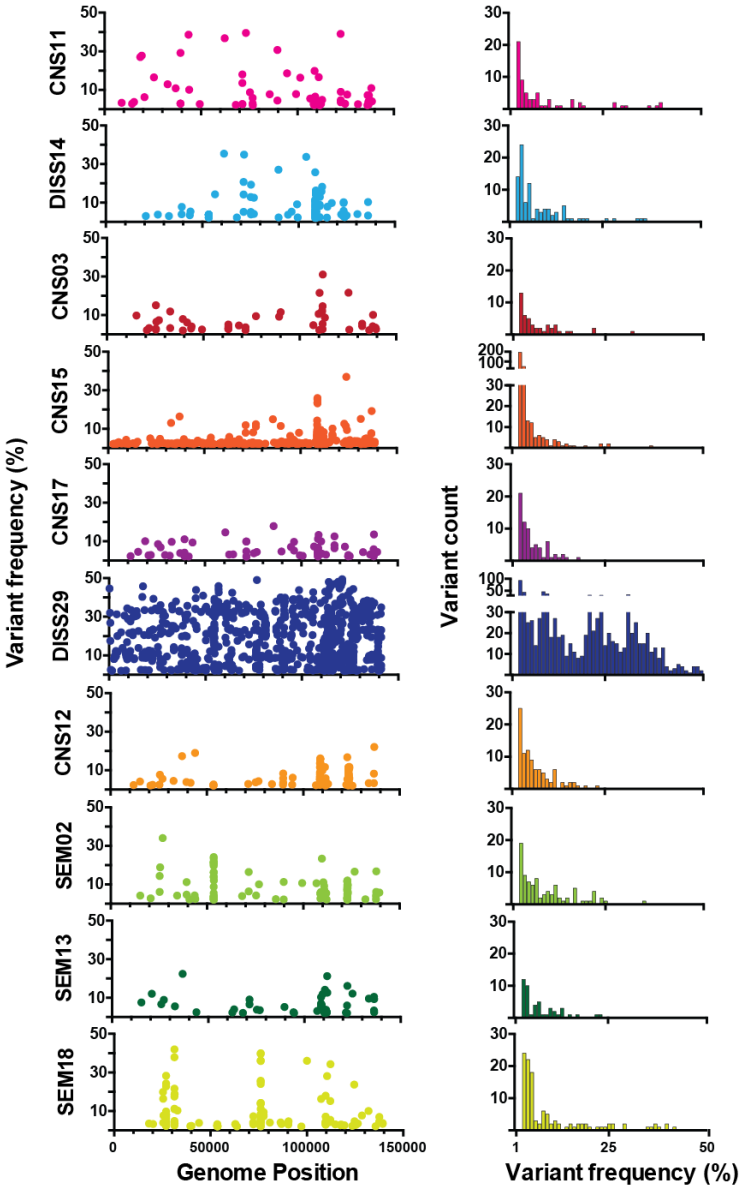

Figure S5. Genome-wide distribution and frequency of minor variants in neonatal HSV-2 isolates.

For each neonatal HSV-2 isolate, the graph on the left plots spatial location in the genome (x-axis) against the frequency at which each minor variant was observed. The plot on the right summarizes the number of minor variants (y-axis height) in binned increments of 1% (x-axis). The data reveal the distinctly different distribution of minor variants in DISS29, and to a lesser extent CNS15, than in other isolates. Color code matches that used in **Figure 6**. See **Table S3** for full list of SNP and INDEL MV position and frequency data.

Supplemental Table S1. Adult HSV-2 genomes (58 total) used for comparative genomic analyses.

Virus Isolate	Country (with location, if available)	GenBank Accession #	References
H1227*	Finland	KY922721	In process
H1229*	Finland	KY922722	In process
H12212*	Finland	KY922726	In process
Uganda.2*	Uganda	KX574906	(1)
Peru.8*	Peru	KX574874	(1)
Zambia.4*	Zambia	KX574883	(1)
HG52*	Scotland	NC_001798	(2)
CtSF-R*	USA	KP334093	(3)
1192*	Wisconsin, USA	KP334095	(3)
SD90e*	Carletonville, South Africa	KF781518	(4, 5)
Peru.1	Peru	KX574860	(1, 6)
Peru.12	Peru	KX574861	(1, 6)
Zambia.3	Zambia	KX574862	(1, 6)
Zimbabwe.2	Zimbabwe	KX574863	(1, 6)
Zambia.2	Zambia	KX574864	(1, 6)
Peru.9	Peru	KX574865	(1, 6)
Peru.11	Peru	KX574866	(1, 6)
Peru.10	Peru	KX574867	(1, 6)
USA.8a	USA	KX574868	(1, 6)
USA.8b	USA	KX574869	(1, 6)
USA.1b	USA	KX574870	(1, 6)
Zimbabwe.1	Zimbabwe	KX574871	(1, 6)
Peru.7	Peru	KX574872	(1, 6)
Peru.2	Peru	KX574873	(1, 6)
Peru.6	Peru	KX574875	(1, 6)
Peru.4	Peru	KX574876	(1, 6)
Peru.3	Peru	KX574877	(1, 6)
Peru.5	Peru	KX574878	(1, 6)
South Africa.2	South Africa	KX574879	(1, 6)
South Africa.3	South Africa	KX574880	(1, 6)
South Africa.4	South Africa	KX574881	(1, 6)
USA.3a	USA	KX574882	(1, 6)
USA.3b	USA	KX574884	(1, 6)
USA.2b	USA	KX574885	(1, 6)
USA.10	USA	KX574886	(1, 6)
USA.4a	USA	KX574887	(1, 6)
USA.7a	USA	KX574888	(1, 6)

Virus Isolate	Country (with location, if available)	GenBank Accession #	References
USA.6a	USA	KX574889	(1, 6)
USA.6b	USA	KX574890	(1, 6)
USA.5a	USA	KX574891	(1, 6)
South Africa.1	South Africa	KX574892	(1, 6)
Kenya.2	Kenya	KX574893	(1, 6)
Kenya.1	Kenya	KX574894	(1, 6)
USA.7b	USA	KX574895	(1, 6)
USA.5b	USA	KX574896	(1, 6)
USA.11	USA	KX574897	(1, 6)
USA.4b	USA	KX574898	(1, 6)
Uganda.1	Uganda	KX574899	(1, 6)
USA.9	USA	KX574901	(1, 6)
Tanzania.1	Tanzania	KX574902	(1, 6)
USA.1a	USA	KX574903	(1, 6)
USA.2a	USA	KX574904	(1, 6)
Zambia.1	Zambia	KX574905	(1, 6)
2011-21400	USA	KX574908	(1, 6)
H12211	Finland	KY922725	In process
H1226	Finland	KY922720	In process
H1421	Finland	KY922723	In process
H1526	Finland	KY922724	In process

*Indicates 10 adult HSV-2 strains used for AA comparisons in Figure 5.

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Table S2. Number of nucleotide and amino acid (AA) differences observed in each set of neonatal or adult HSV-2 genomes.

Gene*	ORF length in NT^	protein length (#AA)	10 Neonatal # NT diff.^	10 Neonatal # AA changes	10 Neonatal %AA diff.	10 Neonatal dN/dS ratio	10 Adult # NT diff.	10 Adult # AA changes	10 Adult %AA diff.	10 Adult dN/dS ratio	58 Adult # NT diff.	58 Adult # AA diff.	58 Adult %AA diff.	58 Adult dN/dS ratio
UL1	675	225	5	2	0.9	**0.7**	3	2	0.9	**2**	10	6	2.7	**1.5**
UL2	1,005	335	7	4	1.2	**1.3**	10	3	0.9	**0.4**	14	5	1.5	**0.6**
UL3	669	223	1	0	0	**0**	2	0	0	**0**	8	3	1.3	**0.6**
UL4	606	202	5	2	1	**0.7**	6	2	1	**0.5**	10	2	1	**0.3**
UL5	2,646	882	18	4	0.5	**0.3**	21	3	0.3	**0.2**	43	11	1.2	**0.3**
UL6	2,037	679	9	3	0.4	**0.5**	6	3	0.4	**1**	26	11	1.6	**0.7**
UL7	891	297	4	1	0.3	**0.3**	5	2	0.7	**0.7**	15	6	2	**0.7**
UL8	2,259	753	13	5	0.7	**0.6**	18	7	0.9	**0.6**	39	18	2.4	**0.9**
UL9	2,616	872	9	3	0.3	**0.5**	14	4	0.5	**0.4**	35	11	1.3	**0.5**
UL10	1,404	468	5	1	0.2	**0.3**	6	1	0.2	**0.2**	19	5	1.1	**0.4**
UL11	291	97	1	0	0	**0**	4	2	2.1	**1**	6	3	3.1	**1**
UL12	1,863	621	6	4	0.6	**2**	14	9	1.4	**1.8**	42	24	3.9	**1.3**
UL13	1,557	519	8	2	0.4	**0.3**	6	2	0.4	**0.5**	20	5	1	**0.3**
UL14	660	220	3	0	0	**0**	2	1	0.5	**1**	3	2	0.9	**2**
UL15	2,205	735	2	1	0.1	**1**	4	1	0.1	**0.3**	20	4	0.5	**0.3**
UL16	1,119	373	4	2	0.5	**1**	5	3	0.8	**1.5**	8	4	1.1	**1**
UL17	2,109	703	13	8	1.1	**1.6**	9	2	0.3	**0.3**	24	12	1.7	**1**
UL18	957	319	4	1	0.3	**0.3**	4	0	0	**0**	12	1	0.3	**0.1**
UL19	4,125	1,375	14	3	0.2	**0.3**	21	6	0.4	**0.4**	41	12	0.9	**0.4**
UL20	669	223	4	3	1.3	**3**	6	5	2.2	**5**	10	7	3.1	**2.3**
UL21	1,599	533	4	3	0.6	**3**	3	2	0.4	**2**	11	7	1.3	**1.8**
UL22	2,517	839	22	7	0.8	**0.5**	20	6	0.7	**0.4**	46	18	2.1	**0.6**
UL23	1,131	377	7	5	1.3	**2.5**	11	6	1.6	**1.2**	20	12	3.2	**1.5**
UL24	846	282	12	3	1.1	**0.3**	14	4	1.4	**0.4**	26	11	3.9	**0.7**
UL25	1,758	586	7	3	0.5	**0.8**	8	1	0.2	**0.1**	25	4	0.7	**0.2**
UL26	1,914	638	11	3	0.5	**0.4**	18	7	1.1	**0.6**	36	16	2.5	**0.8**
UL26.5	990	330	8	2	0.6	**0.3**	11	6	1.8	**1.2**	25	14	4.2	**1.3**
UL27	2,715	905	16	10	1.1	**1.7**	17	8	0.9	**0.9**	39	17	1.9	**0.8**
UL28	2,358	786	14	7	0.9	**1**	11	5	0.6	**0.8**	39	15	1.9	**0.6**
UL29	3,591	1,197	27	8	0.7	**0.4**	32	11	0.9	**0.5**	83	24	2	**0.4**
UL30	3,723	1,241	19	12	1	**1.7**	39	20	1.6	**1.1**	68	35	2.8	**1.1**
UL31	918	306	6	1	0.3	**0.2**	9	2	0.7	**0.3**	18	7	2.3	**0.6**
UL32	1,797	599	13	4	0.7	**0.4**	13	7	1.2	**1.2**	20	8	1.3	**0.7**
UL33	393	131	2	0	0	**0**	1	0	0	**0**	5	1	0.8	**0.3**
UL34	831	277	8	4	1.4	**1**	7	2	0.7	**0.4**	15	5	1.8	**0.5**
UL35	339	113	0	0	0	**0**	2	1	0.9	**0**	6	4	3.5	**2**
UL36	9,369	3,123	65	31	1	**0.9**	73	33	1.1	**0.8**	181	82	2.6	**0.8**
UL37	3,345	1,115	13	8	0.7	**1.6**	27	16	1.4	**1.5**	54	25	2.2	**0.9**
UL38	1,401	467	10	8	1.7	**4**	22	9	1.9	**0.7**	38	15	3.2	**0.7**
UL39	3,429	1,143	64	22	1.9	**0.5**	72	25	2.2	**0.5**	118	49	4.3	**0.7**
UL40	1,014	338	6	2	0.6	**0.5**	4	0	0	**0**	13	3	0.9	**0.3**
UL41	1,479	493	6	1	0.2	**0.2**	13	2	0.4	**0.2**	26	8	1.6	**0.4**
UL42	1,413	471	8	4	0.8	**1**	16	8	1.7	**1**	21	12	2.5	**1.3**

Table S2. Number of nucleotide and amino acid (AA) differences observed in each set of neonatal or adult HSV-2 genomes.

Gene*	ORF length in NT^	protein length (#AA)	10 Neonatal # NT diff.^	10 Neonatal # AA changes	10 Neonatal %AA diff.	10 Neonatal dN/dS ratio	10 Adult # NT diff.	10 Adult # AA changes	10 Adult %AA diff.	10 Adult dN/dS ratio	58 Adult # NT diff.	58 Adult # AA diff.	58 Adult %AA diff.	58 Adult dN/dS ratio
UL43	1,245	415	10	6	1.4	**1.5**	8	6	1.4	**3**	27	17	4.1	**1.7**
UL44	1,443	481	13	6	1.2	**0.9**	12	7	1.5	**1.4**	23	15	3.1	**1.9**
UL45	519	173	1	1	0.6	**0**	3	1	0.6	**0**	6	3	1.7	**1**
UL46	2,169	723	20	9	1.2	**0.8**	18	7	1	**0.6**	40	17	2.4	**0.7**
UL47	2,091	697	14	6	0.9	**0.8**	13	9	1.3	**2.3**	43	20	2.9	**0.9**
UL48	1,473	491	6	3	0.6	**1**	7	2	0.4	**0.4**	18	8	1.6	**0.8**
UL49	903	301	19	7	2.3	**0.6**	17	7	2.3	**0.7**	30	12	4	**0.7**
UL49A	264	88	3	1	1.1	**0.5**	6	2	2.3	**0.5**	8	2	2.3	**0.3**
UL50	1,110	370	8	4	1.1	**1**	7	4	1.1	**1.3**	22	10	2.7	**0.8**
UL51	735	245	6	1	0.4	**0.2**	1	0	0	**0**	11	6	2.4	**1.2**
UL52	3,201	1,067	22	12	1.1	**1.2**	31	11	1	**0.6**	54	25	2.3	**0.9**
UL53	1,017	339	14	8	2.4	**1.3**	16	10	2.9	**1.7**	26	14	4.1	**1.2**
UL54	1,539	513	5	0	0	**0**	7	3	0.6	**0.8**	24	12	2.3	**1**
UL55	561	187	3	1	0.5	**0.5**	2	2	1.1	**0**	9	3	1.6	**0.5**
UL56	708	236	6	4	1.7	**2**	8	5	2.1	**1.7**	19	11	4.7	**1.4**
RL2	2,502	834	43	22	2.6	**1**	33	19	2.3	**1.4**	48	23	2.8	**0.9**
US1	1,245	415	16	5	1.2	**0.5**	14	6	1.4	**0.8**	38	22	5.3	**1.4**
US2	876	292	7	4	1.4	**1.3**	4	3	1	**3**	15	7	2.4	**0.9**
US3	1,446	482	9	4	0.8	**0.8**	7	4	0.8	**1.3**	17	4	0.8	**0.3**
US4	2,100	700	22	13	1.9	**1.4**	40	25	3.6	**1.7**	70	45	6.4	**1.8**
US5	279	93	4	1	1.1	**0.3**	8	4	4.3	**1**	11	6	6.5	**1.2**
US6	1,182	394	7	2	0.5	**0.4**	8	2	0.5	**0.3**	18	5	1.3	**0.4**
US7	1,119	373	10	6	1.6	**1.5**	11	5	1.3	**0.8**	27	14	3.8	**1.1**
US8	1,638	546	12	9	1.6	**3**	9	9	1.6	**0**	28	20	3.7	**2.5**
US8A	441	147	4	3	2	**3**	2	0	0	**0**	7	2	1.4	**0.4**
US9	270	90	2	0	0	**0**	3	1	1.1	**0.5**	7	4	4.4	**1.3**
US10	261	87	7	1	1.1	**0.2**	3	2	2.3	**2**	6	2	2.3	**0.5**
US11	456	152	2	2	1.3	**0**	0	0	0	**0**	136	5	3.3	**0**
US12	909	303	6	4	1.3	**2**	6	0	0	**0**	11	10	3.3	**10**

* See **Table S1** for list of strain names and genome accessions for comparisons of 10 neonatal, 10 adult, and 58 adult HSV-2 genomes.
^ Abbreviations: ORF, open reading frame; NT, nucleotide; AA, amino acid; diff., difference; %, percent; dN/dS, ratio of nonsynonymous to synonymous nucleotide changes

Supplemental Table S4. Viral genetic variations potentially associated with clinical groups.

HSV-2 Gene: Variation	Clinical Group (Isolates w/ Variation)	Predicted effect^	Protein Function	Neurovirulence
gK (UL53): V323M	All CSF Isolates (CNS11, DISS14)	Loss of one TM domain	Glycoprotein at virion and cell surface; interacts with gB and UL20 (1); important for cytoplasmic envelopment, viral egress, and virus-induced cell fusion	gK null virus exhibits poor neuronal spread in culture including decreased retrograde and anterograde transport (2); reduced spread within eye and to nervous system, as well as increased survival, following ocular challenge in mice (3)
gI (US7): R159L + P215S	All CSF Isolates (Both: CNS11,DISS14 R159L only: CNS03, SEM13,CNS15*,DISS29* P215S only: CNS15*,DISS29)	R159L: adds one TM domain; P215S: no change	Glycoprotein at virion and cell surface; forms heterodimer with gE to form viral Fc receptor (4); required for cell-to-cell spread of virus in epithelial and neuronal cells (5, 6);	gI null virus exhibits poor neuronal spread in culture; reduced spread in retina and to retinorecipient areas of the brain following ocular challenge in mice (6)
UL8: R221S	All CSF Isolates (CNS11, DISS14)	No predicted change	Putative primase subunit of helicase primase complex; required for unwinding of viral DNA	None identified
US2: F137L	All CSF Isolates (CNS11, DISS14)	No predicted change	Tegument protein; binds and phosphorylates TAK1 to positively regulate NFkB signaling (7)	Virus containing mutated US2 forms small plaques in culture (8); Mutant virus containing a deletion of both US2 and PK result in decreased death following intracerebral injection in mice (9)
gG (US4): R338L,S442P, E574D	All CSF Isolates (CNS11, DISS14)	No predicted change	Binds and enhances function of chemokines (10); enhances neurotrophin-dependent axonal growth in culture (11)	Virus containing mutated gG results in decreased death following intracerebral injection in mice (8)
UL24: V93A	No CNS Isolates; only found in SEM isolates (SEM02, SEM13, SEM18)	No predicted change	Membrane-associated nuclear protein; associated with dispersal of nucleolin during infection (12, 13)	UL24 null virus forms small plaques in culture (14); decreased viral load in eye and nervous system following ocular challenge in mice (15–17)
UL20: P129L	All DISS Isolates w/ CNS Involved (DISS14, DISS29)	Change in position of three TM domains	Membrane-associated protein interacts with gK and gB (1); required for gK glycosylation, cell surface expression (18), and virus-induced cell fusion (19); important for viral egress (20)	UL20 null virus forms small plaques in culture (19); RNA inhibition of UL20 results in decreased rates of encephalitis following HSV footpad infection in mice (21)

* present as minor variant ^ Prediction by SMART Analysis

References for Supplemental Table S4

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Table S3. Minor variants -- SNPs and INDELS -- detected in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
SEM02	25378	C	T	6.1%	568	134	7	39	15.2%	84.8%	Intergenic	intergenic
SEM02	34462	G	A	4.2%	887	207	39	9	81.3%	18.8%	Intergenic	intergenic
SEM02	39394	C	T	11.2%	20	557	53	20	72.6%	27.4%	Intergenic	intergenic
SEM02	40897	C	T	2.2%	1737	823	39	19	67.2%	32.8%	Intergenic	intergenic
SEM02	53425	G	T	23.5%	199	1260	94	415	18.5%	81.5%	Intergenic	intergenic
SEM02	53426	C	G	21.2%	213	1340	84	370	18.5%	81.5%	Intergenic	intergenic
SEM02	53427	G	A	24.2%	199	1290	101	416	19.5%	80.5%	Intergenic	intergenic
SEM02	53428	C	T	3.7%	311	1719	11	68	13.9%	86.1%	Intergenic	intergenic
SEM02	53433	T	C	5.5%	241	1550	21	83	20.2%	79.8%	Intergenic	intergenic
SEM02	53434	C	G	2.2%	253	1592	9	33	21.4%	78.6%	Intergenic	intergenic
SEM02	53435	G	A	20.3%	172	1243	72	305	19.1%	80.9%	Intergenic	intergenic
SEM02	53436	G	C	21.3%	171	1258	79	313	20.2%	79.8%	Intergenic	intergenic
SEM02	53438	A	C	21.4%	167	1249	77	312	19.8%	80.2%	Intergenic	intergenic
SEM02	53442	G	C	16.3%	182	1244	55	224	19.7%	80.3%	Intergenic	intergenic
SEM02	53444	C	G	13.7%	182	1249	47	181	20.6%	79.4%	Intergenic	intergenic
SEM02	53445	G	C	16.3%	181	1196	52	217	19.3%	80.7%	Intergenic	intergenic
SEM02	53446	T	C	14.2%	179	1180	48	180	21.1%	78.9%	Intergenic	intergenic
SEM02	53455	T	A	8.8%	228	1135	30	103	22.6%	77.4%	Intergenic	intergenic
SEM02	53457	C	A	3.9%	241	1152	12	44	21.4%	78.6%	Intergenic	intergenic
SEM02	53458	T	G	3.7%	244	1104	10	44	18.5%	81.5%	Intergenic	intergenic
SEM02	53459	A	C	2.6%	258	1083	5	32	13.5%	86.5%	Intergenic	intergenic
SEM02	53550	T	C	2.8%	1133	135	26	10	72.2%	27.8%	Intergenic	intergenic
SEM02	53556	A	C	12.0%	1196	82	162	18	90.0%	10.0%	Intergenic	intergenic
SEM02	71632	C	A	6.3%	348	266	30	12	71.4%	28.6%	Intergenic	intergenic
SEM02	108818	C	G	5.3%	1082	72	57	8	87.7%	12.3%	Intergenic	intergenic
SEM02	108852	A	C	2.7%	1076	172	31	4	88.6%	11.4%	Intergenic	intergenic
SEM02	110803	A	C	3.9%	587	500	8	36	18.2%	81.8%	Intergenic	intergenic
SEM02	122812	C	G	3.5%	3501	78	83	47	63.8%	36.2%	Intergenic	intergenic
SEM02	122813	G	T	2.9%	3455	79	71	36	66.4%	33.6%	Intergenic	intergenic
SEM02	122816	G	A	2.0%	3428	141	50	24	67.6%	32.4%	Intergenic	intergenic
SEM02	122817	A	C	2.6%	3263	160	61	31	66.3%	33.7%	Intergenic	intergenic
SEM02	122820	T	G	7.6%	3184	84	161	109	59.6%	40.4%	Intergenic	intergenic
SEM02	122827	G	C	6.9%	3169	150	191	60	76.1%	23.9%	Intergenic	intergenic
SEM02	122828	T	G	8.2%	3078	108	195	96	67.0%	33.0%	Intergenic	intergenic
SEM02	122829	T	G	9.5%	3086	94	204	135	60.2%	39.8%	Intergenic	intergenic
SEM02	122832	T	C	11.7%	3069	119	234	189	55.3%	44.7%	Intergenic	intergenic
SEM02	122838	G	C	2.8%	3287	243	33	69	32.4%	67.6%	Intergenic	intergenic
SEM02	122839	G	C	11.3%	3048	211	246	169	59.3%	40.7%	Intergenic	intergenic
SEM02	122840	C	G	11.6%	3001	97	252	176	58.9%	41.1%	Intergenic	intergenic
SEM02	122993	A	G	8.2%	1361	3563	376	74	83.6%	16.4%	Intergenic	intergenic
SEM02	123000	C	G	9.9%	1191	3669	472	59	88.9%	11.1%	Intergenic	intergenic
SEM02	123004	G	C	6.1%	1222	3755	279	45	86.1%	13.9%	Intergenic	intergenic
SEM02	123006	C	G	5.3%	1029	3785	240	36	87.0%	13.0%	Intergenic	intergenic
SEM02	132214	A	G	2.3%	69	574	12	3	80.0%	20.0%	Intergenic	intergenic
CNS03	21910	T	G	3.3%	733	6	4	21	16.0%	84.0%	Intergenic	intergenic
CNS03	25388	C	T	2.9%	345	192	8	8	50.0%	50.0%	Intergenic	intergenic
CNS03	25399	C	T	2.4%	303	379	8	9	47.1%	52.9%	Intergenic	intergenic
CNS03	32756	C	T	3.3%	445	727	4	36	10.0%	90.0%	Intergenic	intergenic
CNS03	39363	C	T	2.1%	570	512	16	7	69.6%	30.4%	Intergenic	intergenic
CNS03	43652	T	G	3.0%	300	22	9	1	90.0%	10.0%	Intergenic	intergenic
CNS03	62950	C	T	2.8%	35	245	7	1	87.5%	12.5%	Non-synonymous mutation	UL36
CNS03	62952	A	G	2.9%	34	238	7	1	87.5%	12.5%	Synonymous mutation	UL36
CNS03	62955	T	C	3.8%	33	223	9	1	90.0%	10.0%	Synonymous mutation	UL36
CNS03	62957	C	G	5.2%	34	205	11	2	84.6%	15.4%	Non-synonymous mutation	UL36
CNS03	111900	A	C	2.7%	279	253	7	8	46.7%	53.3%	Intergenic	intergenic
CNS03	111904	A	C	5.4%	249	254	8	21	27.6%	72.4%	Intergenic	intergenic
CNS03	125251	C	G	21.6%	297	47	13	82	13.7%	86.3%	Intergenic	intergenic
CNS03	132366	G	A	5.6%	49	181	5	9	35.7%	64.3%	Intergenic	intergenic
CNS03	132367	G	A	4.3%	54	257	10	4	71.4%	28.6%	Intergenic	intergenic
CNS11	14287	C	T	2.9%	1234	851	36	26	58.1%	41.9%	Non-synonymous mutation	UL10
CNS11	18524	A	G	27.1%	1162	823	439	299	59.5%	40.5%	Non-synonymous mutation	UL13
CNS11	19290	T	C	27.8%	1239	739	471	291	61.8%	38.2%	Synonymous mutation	UL14
CNS11	36909	C	A	10.9%	1309	726	158	91	63.5%	36.5%	Synonymous mutation	UL22
CNS11	39354	C	T	3.0%	518	567	24	9	72.7%	27.3%	Intergenic	intergenic
CNS11	62310	G	A	36.8%	222	684	143	385	27.1%	72.9%	Synonymous mutation	UL36
CNS11	71447	A	C	2.7%	160	134	2	6	25.0%	75.0%	Intergenic	intergenic
CNS11	71464	C	A	2.7%	73	181	6	1	85.7%	14.3%	Intergenic	intergenic
CNS11	71465	C	A	13.6%	60	140	15	20	42.9%	57.1%	Intergenic	intergenic
CNS11	71466	A	C	18.1%	50	154	16	29	35.6%	64.4%	Intergenic	intergenic
CNS11	73273	C	T	39.6%	838	496	535	339	61.2%	38.8%	Non-synonymous mutation	UL37
CNS11	76821	T	C	3.1%	589	201	5	20	20.0%	80.0%	Intergenic	intergenic
CNS11	76825	A	C	5.8%	421	191	19	19	50.0%	50.0%	Intergenic	intergenic
CNS11	76847	A	C	2.0%	98	291	7	1	87.5%	12.5%	Intergenic	intergenic
CNS11	101493	G	A	16.4%	1004	888	203	167	54.9%	45.1%	Non-synonymous mutation	UL52
CNS11	108337	T	C	2.8%	0	242	3	4	42.9%	57.1%	Intergenic	intergenic
CNS11	108720	C	G	5.4%	290	39	17	2	89.5%	10.5%	Intergenic	intergenic
CNS11	108723	C	G	2.3%	283	48	5	3	62.5%	37.5%	Intergenic	intergenic
CNS11	108725	A	G	2.3%	247	48	6	1	85.7%	14.3%	Intergenic	intergenic
CNS11	108729	C	G	2.2%	250	62	4	3	57.1%	42.9%	Intergenic	intergenic
CNS11	108870	C	G	2.3%	749	164	12	9	57.1%	42.9%	Intergenic	intergenic
CNS11	109299	G	A	3.0%	19	397	6	7	46.2%	53.8%	Intergenic	intergenic
CNS11	112775	G	T	4.9%	988	1736	56	83	40.3%	59.7%	Synonymous mutation	RL2
CNS11	122339	T	C	39.1%	630	744	467	415	52.9%	47.1%	Synonymous mutation	RS1
CNS11	122445	G	T	9.1%	51	163	19	3	86.4%	13.6%	Intergenic	intergenic
CNS11	122447	G	T	3.6%	25	232	8	2	80.0%	20.0%	Intergenic	intergenic
CNS11	122448	G	T	4.7%	18	265	7	7	50.0%	50.0%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
CNS11	131364	G	A	2.7%	55	306	1	9	10.0%	90.0%	Intergenic	intergenic
CNS11	136257	C	A	2.1%	947	883	22	18	55.0%	45.0%	Synonymous mutation	US8A
CNS11	136391	G	A	7.3%	153	1005	13	78	14.3%	85.7%	Intergenic	intergenic
CNS11	136861	G	T	2.5%	650	726	14	21	40.0%	60.0%	Intergenic	intergenic
CNS12	11965	G	A	2.4%	1402	920	31	25	55.0%	45.0%	Non-synonymous mutation	UL9
CNS12	21908	T	G	2.5%	758	145	7	16	30.0%	70.0%	Intergenic	intergenic
CNS12	53262	C	G	2.3%	1916	337	10	43	19.0%	81.0%	Intergenic	intergenic
CNS12	53420	T	G	2.1%	2656	1457	10	79	11.0%	89.0%	Intergenic	intergenic
CNS12	53422	T	C	2.7%	2648	1404	15	101	13.0%	87.0%	Intergenic	intergenic
CNS12	53424	A	T	2.1%	2568	1456	11	78	12.0%	88.0%	Intergenic	intergenic
CNS12	83833	T	C	2.9%	163	567	19	3	86.0%	14.0%	Intergenic	intergenic
CNS12	89578	T	C	2.5%	206	407	5	11	31.0%	69.0%	Intergenic	intergenic
CNS12	89605	C	A	3.6%	260	577	10	22	31.0%	69.0%	Intergenic	intergenic
CNS12	94275	G	T	2.6%	715	199	3	21	13.0%	88.0%	Intergenic	intergenic
CNS12	106607	C	A	2.3%	303	521	12	7	63.0%	37.0%	Intergenic	intergenic
CNS12	108735	G	A	11.5%	286	68	30	16	65.0%	35.0%	Intergenic	intergenic
CNS12	108737	C	G	3.9%	323	64	2	14	13.0%	88.0%	Intergenic	intergenic
CNS12	108739	C	G	7.9%	291	84	25	7	78.0%	22.0%	Intergenic	intergenic
CNS12	108740	C	G	6.4%	308	71	7	19	27.0%	73.0%	Intergenic	intergenic
CNS12	108741	A	G	8.8%	243	84	23	9	72.0%	28.0%	Intergenic	intergenic
CNS12	108742	C	G	13.7%	258	69	27	25	52.0%	48.0%	Intergenic	intergenic
CNS12	108743	A	G	6.8%	254	86	20	5	80.0%	20.0%	Intergenic	intergenic
CNS12	108744	C	A	5.1%	252	92	15	4	79.0%	21.0%	Intergenic	intergenic
CNS12	108745	C	G	5.2%	248	82	4	15	21.0%	79.0%	Intergenic	intergenic
CNS12	108747	A	G	5.6%	222	59	14	4	78.0%	22.0%	Intergenic	intergenic
CNS12	108751	C	G	4.1%	223	95	2	12	14.0%	86.0%	Intergenic	intergenic
CNS12	108754	C	G	2.5%	203	113	4	4	50.0%	50.0%	Intergenic	intergenic
CNS12	108763	G	C	14.0%	130	100	34	4	89.0%	11.0%	Intergenic	intergenic
CNS12	108876	C	G	3.7%	362	129	10	9	53.0%	47.0%	Intergenic	intergenic
CNS12	108877	C	G	4.1%	363	135	11	11	50.0%	50.0%	Intergenic	intergenic
CNS12	108878	C	G	2.7%	362	137	11	4	73.0%	27.0%	Intergenic	intergenic
CNS12	108880	C	G	3.1%	402	155	10	8	56.0%	44.0%	Intergenic	intergenic
CNS12	108887	A	G	15.8%	369	121	55	38	59.0%	41.0%	Intergenic	intergenic
CNS12	108890	G	C	16.2%	394	168	68	41	62.0%	38.0%	Intergenic	intergenic
CNS12	108892	C	G	15.8%	410	194	76	39	66.0%	34.0%	Intergenic	intergenic
CNS12	111255	G	C	2.3%	238	65	2	5	29.0%	71.0%	Intergenic	intergenic
CNS12	111625	A	C	2.1%	191	501	2	13	13.0%	87.0%	Intergenic	intergenic
CNS12	111629	A	C	2.8%	185	514	4	16	20.0%	80.0%	Intergenic	intergenic
CNS12	112864	A	C	3.0%	127	779	25	3	89.0%	11.0%	Intergenic	intergenic
CNS12	122917	T	G	5.5%	97	456	27	5	84.0%	16.0%	Intergenic	intergenic
CNS12	123410	C	G	2.7%	3209	113	76	16	83.0%	17.0%	Intergenic	intergenic
CNS12	123411	G	A	2.7%	3156	117	84	9	90.0%	10.0%	Intergenic	intergenic
CNS12	123415	A	C	4.9%	2891	128	134	22	86.0%	14.0%	Intergenic	intergenic
CNS12	123416	G	T	4.8%	2967	119	133	24	85.0%	15.0%	Intergenic	intergenic
CNS12	123418	T	G	6.4%	2929	124	170	38	82.0%	18.0%	Intergenic	intergenic
CNS12	123425	G	C	7.1%	2980	148	210	34	86.0%	14.0%	Intergenic	intergenic
CNS12	123426	T	G	8.6%	2875	135	236	51	82.0%	18.0%	Intergenic	intergenic
CNS12	123427	T	G	10.0%	2892	128	260	77	77.0%	23.0%	Intergenic	intergenic
CNS12	123430	T	C	11.0%	2947	159	297	88	77.0%	23.0%	Intergenic	intergenic
CNS12	123432	C	A	2.1%	3264	221	34	41	45.0%	55.0%	Intergenic	intergenic
CNS12	123436	G	C	4.1%	3211	216	53	95	36.0%	64.0%	Intergenic	intergenic
CNS12	123437	G	C	11.1%	2950	253	318	83	79.0%	21.0%	Intergenic	intergenic
CNS12	123438	C	G	11.6%	2888	128	330	93	78.0%	22.0%	Intergenic	intergenic
CNS12	123591	A	G	7.9%	1923	3195	374	73	84.0%	16.0%	Intergenic	intergenic
CNS12	123598	C	G	9.7%	1726	3337	483	62	89.0%	11.0%	Intergenic	intergenic
CNS12	123602	G	C	7.4%	1591	3424	352	50	88.0%	12.0%	Intergenic	intergenic
CNS12	123604	C	G	6.8%	1407	3431	309	46	87.0%	13.0%	Intergenic	intergenic
CNS12	123607	G	T	3.3%	1107	3478	137	26	84.0%	16.0%	Intergenic	intergenic
CNS12	123608	G	A	4.8%	1360	3468	222	24	90.0%	10.0%	Intergenic	intergenic
CNS12	123921	C	A	11.8%	88	465	20	54	27.0%	73.0%	Intergenic	intergenic
CNS12	123934	C	A	11.2%	85	191	12	23	34.0%	66.0%	Intergenic	intergenic
CNS12	123999	A	C	5.8%	128	1	7	1	88.0%	13.0%	Intergenic	intergenic
CNS12	124067	A	C	3.0%	395	28	10	3	77.0%	23.0%	Intergenic	intergenic
CNS12	136687	G	T	3.4%	340	371	5	20	20.0%	80.0%	Intergenic	intergenic
SEM13	36678	G	T	22.4%	262	579	67	176	28.0%	72.4%	Synonymous mutation	UL22
SEM13	62504	C	G	2.3%	10	325	6	2	75.0%	25.0%	Non-synonymous mutation	UL36
SEM13	63458	C	G	3.9%	73	147	1	8	11.0%	88.9%	Non-synonymous mutation	UL36
SEM13	71465	C	A	9.1%	99	155	11	16	41.0%	59.3%	Intergenic	intergenic
SEM13	71466	A	C	6.5%	90	211	11	10	52.0%	47.6%	Intergenic	intergenic
SEM13	76818	A	C	3.6%	190	215	2	13	13.0%	86.7%	Intergenic	intergenic
SEM13	94282	G	T	2.5%	625	155	3	17	15.0%	85.0%	Intergenic	intergenic
SEM13	106636	T	C	3.2%	61	584	18	3	86.0%	14.3%	Intergenic	intergenic
SEM13	108636	G	C	3.8%	730	62	27	4	87.0%	12.9%	Intergenic	intergenic
SEM13	108637	G	A	10.3%	667	54	75	9	89.0%	10.7%	Intergenic	intergenic
SEM13	108642	G	C	6.4%	737	72	51	7	88.0%	12.1%	Intergenic	intergenic
SEM13	108643	G	C	7.0%	713	66	45	18	71.0%	28.6%	Intergenic	intergenic
SEM13	110586	C	A	3.8%	330	270	21	3	88.0%	12.5%	Intergenic	intergenic
SEM13	110630	C	T	2.8%	149	297	7	6	54.0%	46.2%	Intergenic	intergenic
SEM13	111186	C	G	3.7%	178	46	8	1	89.0%	11.1%	Intergenic	intergenic
SEM13	111189	C	G	3.3%	192	37	6	2	75.0%	25.0%	Intergenic	intergenic
SEM13	111647	T	C	2.5%	34	622	14	3	82.0%	17.7%	Intergenic	intergenic
SEM13	122123	T	G	2.0%	296	237	9	2	82.0%	18.2%	Intergenic	intergenic
SEM13	136113	G	C	3.4%	577	563	17	23	43.0%	57.5%	Intergenic	intergenic
SEM13	136231	G	C	2.4%	460	258	3	15	17.0%	83.3%	Intergenic	intergenic
DISS14	39359	C	T	7.8%	13	519	40	5	88.9%	11.1%	Intergenic	intergenic
DISS14	53386	T	G	2.3%	3385	1612	15	106	12.4%	87.6%	Intergenic	intergenic
DISS14	53393	T	G	2.1%	3305	1835	16	96	14.3%	85.7%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS14	53401	A	G	3.8%	3140	1772	19	177	9.7%	90.3%	Intergenic	intergenic
DISS14	53408	C	G	3.7%	3106	1772	18	170	9.6%	90.4%	Intergenic	intergenic
DISS14	53410	G	C	3.7%	3086	1783	18	169	9.6%	90.4%	Intergenic	intergenic
DISS14	53411	C	G	3.7%	3089	1784	18	169	9.6%	90.4%	Intergenic	intergenic
DISS14	53412	C	T	3.8%	3081	1780	19	171	10.0%	90.0%	Intergenic	intergenic
DISS14	56589	C	T	14.3%	1529	1027	258	169	60.4%	39.6%	Synonymous mutation	UL30
DISS14	61295	C	T	35.4%	851	548	439	329	57.2%	42.8%	Synonymous mutation	UL34
DISS14	71435	C	A	5.2%	84	124	10	2	83.3%	16.7%	Intergenic	intergenic
DISS14	71436	C	A	20.7%	73	91	23	23	50.0%	50.0%	Intergenic	intergenic
DISS14	71437	A	C	14.2%	86	125	19	16	54.3%	45.7%	Intergenic	intergenic
DISS14	71708	C	G	34.9%	895	432	471	240	66.2%	33.8%	Synonymous mutation	UL37
DISS14	75091	G	T	12.9%	288	440	71	37	65.7%	34.3%	Intergenic	intergenic
DISS14	75299	A	C	3.9%	425	272	23	5	82.1%	17.9%	Intergenic	intergenic
DISS14	75305	A	C	5.1%	319	333	8	27	22.9%	77.1%	Intergenic	intergenic
DISS14	76797	T	C	4.1%	461	144	3	23	11.5%	88.5%	Intergenic	intergenic
DISS14	76801	A	C	12.6%	261	114	29	25	53.7%	46.3%	Intergenic	intergenic
DISS14	96367	G	C	5.3%	14	148	6	3	66.7%	33.3%	Intergenic	intergenic
DISS14	103979	C	G	33.7%	944	421	466	229	67.1%	32.9%	Synonymous mutation	UL53
DISS14	108618	A	C	10.2%	88	15	10	2	83.3%	16.7%	Intergenic	intergenic
DISS14	108622	C	G	5.2%	107	17	6	1	85.7%	14.3%	Intergenic	intergenic
DISS14	108635	G	C	25.7%	113	19	48	6	88.9%	11.1%	Intergenic	intergenic
DISS14	108641	C	T	67.0%	59	12	137	15	90.1%	9.9%	Intergenic	intergenic
DISS14	108645	C	T	10.9%	199	28	15	14	51.7%	48.3%	Intergenic	intergenic
DISS14	108648	C	G	4.2%	228	43	9	3	75.0%	25.0%	Intergenic	intergenic
DISS14	108649	C	G	11.5%	219	31	18	15	54.5%	45.5%	Intergenic	intergenic
DISS14	108650	G	A	5.5%	233	37	12	4	75.0%	25.0%	Intergenic	intergenic
DISS14	108651	C	G	3.0%	239	46	7	2	77.8%	22.2%	Intergenic	intergenic
DISS14	108652	G	A	4.9%	243	32	11	4	73.3%	26.7%	Intergenic	intergenic
DISS14	108654	C	A	6.0%	245	29	8	11	42.1%	57.9%	Intergenic	intergenic
DISS14	108656	C	G	7.1%	245	38	14	9	60.9%	39.1%	Intergenic	intergenic
DISS14	108657	C	G	3.7%	269	41	2	10	16.7%	83.3%	Intergenic	intergenic
DISS14	108658	G	A	5.8%	268	42	8	11	42.1%	57.9%	Intergenic	intergenic
DISS14	108662	C	G	2.3%	287	50	4	4	50.0%	50.0%	Intergenic	intergenic
DISS14	108663	C	G	2.0%	290	49	3	4	42.9%	57.1%	Intergenic	intergenic
DISS14	108664	G	C	9.1%	282	33	13	19	40.6%	59.4%	Intergenic	intergenic
DISS14	108669	A	G	3.0%	302	49	6	5	54.5%	45.5%	Intergenic	intergenic
DISS14	108672	G	A	3.5%	316	39	3	10	23.1%	76.9%	Intergenic	intergenic
DISS14	108677	G	C	2.8%	330	42	6	5	54.5%	45.5%	Intergenic	intergenic
DISS14	108917	C	T	2.8%	522	174	18	2	90.0%	10.0%	Intergenic	intergenic
DISS14	108924	C	G	3.3%	576	210	24	4	85.7%	14.3%	Intergenic	intergenic
DISS14	108925	C	G	5.4%	560	209	35	11	76.1%	23.9%	Intergenic	intergenic
DISS14	108926	C	G	3.4%	578	211	24	6	80.0%	20.0%	Intergenic	intergenic
DISS14	108928	C	G	3.5%	647	233	25	7	78.1%	21.9%	Intergenic	intergenic
DISS14	108935	A	G	14.8%	620	199	113	31	78.5%	21.5%	Intergenic	intergenic
DISS14	108938	G	C	16.3%	636	259	144	31	82.3%	17.7%	Intergenic	intergenic
DISS14	108940	C	G	14.8%	680	292	136	35	79.5%	20.5%	Intergenic	intergenic
DISS14	110920	G	C	8.9%	129	44	3	14	17.6%	82.4%	Intergenic	intergenic
DISS14	110924	C	T	8.2%	116	75	2	15	11.8%	88.2%	Intergenic	intergenic
DISS14	110925	C	T	2.5%	121	155	2	5	28.6%	71.4%	Intergenic	intergenic
DISS14	122084	G	T	3.9%	674	453	36	10	78.3%	21.7%	Intergenic	intergenic
DISS14	123378	G	C	9.8%	116	36	2	15	11.8%	88.2%	Intergenic	intergenic
DISS14	123381	G	T	5.7%	129	32	1	9	10.0%	90.0%	Intergenic	intergenic
DISS14	123382	G	T	10.2%	133	25	2	16	11.1%	88.9%	Intergenic	intergenic
DISS14	130553	A	G	4.1%	11	506	8	14	36.4%	63.6%	Intergenic	intergenic
DISS14	136049	G	T	3.3%	648	574	14	27	34.1%	65.9%	Intergenic	intergenic
CNS15	2643	C	T	2.1%	2550	949	53	23	69.7%	30.3%	Synonymous mutation	UL4
CNS15	3009	T	G	2.2%	3113	1370	68	32	68.0%	32.0%	Synonymous mutation	UL4
CNS15	3504	G	A	2.2%	713	2821	18	61	22.8%	77.2%	Synonymous mutation	UL5
CNS15	4415	C	T	2.2%	3021	2071	72	41	63.7%	36.3%	Non-synonymous mutation	UL5
CNS15	4545	A	G	2.4%	3281	1579	79	39	66.9%	33.1%	Synonymous mutation	UL5
CNS15	4780	G	T	2.4%	2870	2339	71	57	55.5%	44.5%	Non-synonymous mutation	UL5
CNS15	4830	A	G	2.5%	3069	2047	80	50	61.5%	38.5%	Synonymous mutation	UL5
CNS15	5490	T	G	3.1%	1303	2088	46	61	43.0%	57.0%	Synonymous mutation	UL5
CNS15	6066	A	G	2.6%	3403	890	85	29	74.6%	25.4%	Synonymous mutation	UL6
CNS15	7009	C	G	2.3%	335	1547	10	34	22.7%	77.3%	Non-synonymous mutation	UL6
CNS15	8913	T	C	2.3%	2219	142	48	7	87.3%	12.7%	Intergenic	intergenic
CNS15	9365	T	C	4.3%	648	2498	84	56	60.0%	40.0%	Non-synonymous mutation	UL8
CNS15	10578	C	G	2.1%	2896	2288	71	40	64.0%	36.0%	Non-synonymous mutation	UL8
CNS15	10617	A	G	2.2%	2662	2387	64	51	55.7%	44.3%	Synonymous mutation	UL8
CNS15	13032	C	G	2.6%	2794	2092	80	49	62.0%	38.0%	Non-synonymous mutation	UL9
CNS15	14311	C	G	2.1%	2744	1526	61	31	66.3%	33.7%	Synonymous mutation	UL10
CNS15	14512	C	G	2.7%	3021	2308	88	61	59.1%	40.9%	Synonymous mutation	UL10
CNS15	21911	T	G	5.1%	620	109	24	15	61.5%	38.5%	Intergenic	intergenic
CNS15	22818	C	A	2.3%	2198	2619	46	68	40.4%	59.6%	Non-synonymous mutation	UL17
CNS15	22819	G	C	2.3%	2191	2624	47	67	41.2%	58.8%	Non-synonymous mutation	UL17
CNS15	22820	T	G	2.5%	2090	2626	49	71	40.8%	59.2%	Synonymous mutation	UL17
CNS15	23895	A	C	2.8%	2551	2541	74	74	50.0%	50.0%	Non-synonymous mutation	UL17
CNS15	23898	G	T	2.6%	2653	2627	66	74	47.1%	52.9%	Non-synonymous mutation	UL17
CNS15	26705	C	A	2.6%	2659	1660	75	42	64.1%	35.9%	Intergenic	intergenic
CNS15	26896	C	T	2.5%	2378	2063	65	52	55.6%	44.4%	Intergenic	intergenic
CNS15	28866	T	C	2.6%	3218	1951	83	57	59.3%	40.7%	Synonymous mutation	UL19
CNS15	30504	A	G	2.3%	2904	2734	65	68	48.9%	51.1%	Synonymous mutation	UL19
CNS15	31182	G	A	2.2%	3024	1848	78	33	70.3%	29.7%	Intergenic	intergenic
CNS15	31208	G	A	2.4%	2853	1942	75	42	64.1%	35.9%	Intergenic	intergenic
CNS15	32741	C	T	2.4%	601	1116	8	34	19.0%	81.0%	Intergenic	intergenic
CNS15	33586	T	C	2.3%	2660	2218	59	55	51.8%	48.2%	Non-synonymous mutation	UL21
CNS15	34456	A	G	2.1%	1918	1366	43	28	60.6%	39.4%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
CNS15	35067	G	A	2.0%	3235	1815	68	37	64.8%	35.2%	Synonymous mutation	UL22
CNS15	35224	A	G	2.4%	3176	2352	85	53	61.6%	38.4%	Non-synonymous mutation	UL22
CNS15	35775	G	A	2.7%	2937	2622	71	82	46.4%	53.6%	Synonymous mutation	UL22
CNS15	36726	T	C	2.6%	1038	2864	25	79	24.0%	76.0%	Synonymous mutation	UL22
CNS15	38470	C	T	2.2%	3634	2421	68	67	50.4%	49.6%	Non-synonymous mutation	UL23
CNS15	39182	T	G	2.6%	1033	2227	41	47	46.6%	53.4%	Synonymous mutation	UL24
CNS15	39379	C	T	3.1%	46	1146	32	6	84.2%	15.8%	Intergenic	intergenic
CNS15	39495	A	C	2.2%	1427	1053	33	23	58.9%	41.1%	Intergenic	intergenic
CNS15	41542	T	C	2.9%	2003	2115	74	48	60.7%	39.3%	Intergenic	intergenic
CNS15	42646	G	A	2.3%	3013	1892	74	43	63.2%	36.8%	Non-synonymous mutation	UL26
CNS15	43310	T	C	2.6%	24	580	2	14	12.5%	87.5%	Synonymous mutation	UL26
CNS15	44442	G	T	2.0%	2605	2108	57	41	58.2%	41.8%	Synonymous mutation	UL27
CNS15	45839	A	G	2.5%	2976	2099	83	47	63.8%	36.2%	Synonymous mutation	UL27
CNS15	46478	T	C	3.2%	330	1389	15	41	26.8%	73.2%	Non-synonymous mutation	UL27
CNS15	46510	T	C	2.9%	201	1097	8	31	20.5%	79.5%	Non-synonymous mutation	UL27
CNS15	46543	T	C	3.9%	111	784	6	30	16.7%	83.3%	Non-synonymous mutation	UL27
CNS15	47938	A	G	3.0%	378	1516	13	45	22.4%	77.6%	Synonymous mutation	UL28
CNS15	49032	A	G	2.8%	1990	1795	64	44	59.3%	40.7%	Non-synonymous mutation	UL28
CNS15	49223	C	T	2.1%	159	1327	4	28	12.5%	87.5%	Intergenic	intergenic
CNS15	51185	T	G	2.2%	3284	1813	78	35	69.0%	31.0%	Non-synonymous mutation	UL29
CNS15	51952	T	C	2.9%	2885	2270	91	62	59.5%	40.5%	Synonymous mutation	UL29
CNS15	52429	T	G	2.5%	3044	2126	74	61	54.8%	45.2%	Synonymous mutation	UL29
CNS15	52570	T	C	2.4%	3291	1781	89	38	70.1%	29.9%	Synonymous mutation	UL29
CNS15	52603	T	C	2.5%	2979	1944	89	39	69.5%	30.5%	Synonymous mutation	UL29
CNS15	53046	G	A	2.4%	1969	1477	55	29	65.5%	34.5%	Intergenic	intergenic
CNS15	53257	G	C	4.0%	3396	468	119	41	74.4%	25.6%	Intergenic	intergenic
CNS15	53340	T	C	5.5%	2886	269	117	66	63.9%	36.1%	Intergenic	intergenic
CNS15	53385	A	G	2.4%	3896	3074	106	62	63.1%	36.9%	Intergenic	intergenic
CNS15	53419	T	C	2.2%	4717	3041	29	148	16.4%	83.6%	Intergenic	intergenic
CNS15	54881	G	C	2.2%	2417	2295	61	43	58.7%	41.3%	Synonymous mutation	UL30
CNS15	57805	T	G	2.3%	2217	2381	58	48	54.7%	45.3%	Synonymous mutation	UL31
CNS15	58012	T	C	2.3%	3010	1822	66	47	58.4%	41.6%	Synonymous mutation	UL31
CNS15	58808	A	G	2.8%	460	1919	24	45	34.8%	65.2%	Synonymous mutation	UL32
CNS15	58844	C	G	2.8%	333	1700	20	38	34.5%	65.5%	Synonymous mutation	UL32
CNS15	58870	G	T	2.9%	274	1488	16	36	30.8%	69.2%	Non-synonymous mutation	UL32
CNS15	60036	A	G	2.5%	2955	655	75	18	80.6%	19.4%	Non-synonymous mutation	UL32
CNS15	60113	A	G	2.6%	547	1885	20	46	30.3%	69.7%	Synonymous mutation	UL33
CNS15	62491	C	A	2.2%	75	500	6	7	46.2%	53.8%	Synonymous mutation	UL36
CNS15	62494	G	C	2.1%	77	472	5	7	41.7%	58.3%	Synonymous mutation	UL36
CNS15	62496	G	T	2.1%	73	453	5	6	45.5%	54.5%	Non-synonymous mutation	UL36
CNS15	62501	T	G	2.3%	83	371	4	7	36.4%	63.6%	Non-synonymous mutation	UL36
CNS15	62954	C	T	5.0%	249	168	12	10	54.5%	45.5%	Non-synonymous mutation	UL36
CNS15	62963	A	G	5.8%	252	141	15	9	62.5%	37.5%	Non-synonymous mutation	UL36
CNS15	63223	C	T	3.1%	1384	132	38	10	79.2%	20.8%	Synonymous mutation	UL36
CNS15	63421	A	G	2.2%	1942	652	49	10	83.1%	16.9%	Synonymous mutation	UL32
CNS15	63637	C	T	2.2%	2159	917	60	9	87.0%	13.0%	Synonymous mutation	UL36
CNS15	64732	T	C	2.7%	2782	2380	76	68	52.8%	47.2%	Synonymous mutation	UL36
CNS15	65065	G	A	2.5%	2846	2354	73	58	55.7%	44.3%	Synonymous mutation	UL36
CNS15	65487	T	C	3.5%	2740	1748	84	77	52.2%	47.8%	Non-synonymous mutation	UL36
CNS15	65572	C	G	2.7%	2335	2099	52	72	41.9%	58.1%	Non-synonymous mutation	UL36
CNS15	65760	G	A	2.4%	2764	2193	73	48	60.3%	39.7%	Non-synonymous mutation	UL36
CNS15	67891	A	G	2.3%	2384	219	52	9	85.2%	14.8%	Synonymous mutation	UL37
CNS15	68042	G	C	2.3%	2710	1462	75	21	78.1%	21.9%	Non-synonymous mutation	UL36
CNS15	69534	T	C	2.4%	3207	2424	70	69	50.4%	49.6%	Non-synonymous mutation	UL36
CNS15	70222	T	C	2.5%	2435	615	60	19	75.9%	24.1%	Synonymous mutation	UL37
CNS15	70347	G	C	2.0%	2280	2188	57	36	61.3%	38.7%	Non-synonymous mutation	UL36
CNS15	70444	T	G	2.7%	2261	2107	65	55	54.2%	45.8%	Synonymous mutation	UL36
CNS15	71468	C	A	8.0%	766	430	79	27	74.5%	25.5%	Intergenic	intergenic
CNS15	71469	A	C	2.1%	789	500	22	5	81.5%	18.5%	Intergenic	intergenic
CNS15	72036	A	G	2.1%	2889	2132	62	43	59.0%	41.0%	Non-synonymous mutation	UL37
CNS15	73644	G	A	2.6%	2970	2003	80	53	60.2%	39.8%	Non-synonymous mutation	UL37
CNS15	74825	T	C	2.1%	2254	2310	52	47	52.5%	47.5%	Synonymous mutation	UL37
CNS15	75002	A	C	2.4%	2029	1645	60	29	67.4%	32.6%	Intergenic	intergenic
CNS15	75119	G	T	2.9%	850	1543	30	42	41.7%	58.3%	Intergenic	intergenic
CNS15	75877	C	T	2.3%	2420	822	63	13	82.9%	17.1%	Non-synonymous mutation	UL38
CNS15	76071	C	T	2.5%	2446	2322	66	55	54.5%	45.5%	Non-synonymous mutation	UL38
CNS15	76825	A	C	12.4%	271	252	10	64	13.5%	86.5%	Intergenic	intergenic
CNS15	76829	T	C	11.1%	132	250	15	47	24.2%	75.8%	Intergenic	intergenic
CNS15	77170	T	C	2.4%	3212	1191	75	31	70.8%	29.2%	Intergenic	intergenic
CNS15	77654	C	G	2.4%	1442	2348	38	54	41.3%	58.7%	Non-synonymous mutation	UL39
CNS15	78105	C	T	2.3%	1652	1584	42	33	56.0%	44.0%	Synonymous mutation	UL39
CNS15	78456	A	C	2.4%	2707	1176	63	32	66.3%	33.7%	Synonymous mutation	UL39
CNS15	78655	G	A	2.2%	3048	2297	66	52	55.9%	44.1%	Non-synonymous mutation	UL39
CNS15	78663	C	T	2.3%	3021	2290	73	54	57.5%	42.5%	Synonymous mutation	UL39
CNS15	81685	G	A	2.3%	1740	1516	44	32	57.9%	42.1%	Synonymous mutation	UL40
CNS15	86214	T	C	2.9%	2389	1691	77	44	63.6%	36.4%	Synonymous mutation	UL43
CNS15	87536	C	A	2.6%	530	1280	16	33	32.7%	67.3%	Non-synonymous mutation	UL44
CNS15	87681	T	C	2.2%	1606	1115	34	26	56.7%	43.3%	Non-synonymous mutation	UL44
CNS15	87819	G	A	2.5%	1673	1200	47	26	64.4%	35.6%	Non-synonymous mutation	UL44
CNS15	88345	C	T	2.2%	2832	2329	59	55	51.8%	48.2%	Synonymous mutation	UL44
CNS15	89718	G	A	3.2%	964	853	35	26	57.4%	42.6%	Intergenic	intergenic
CNS15	90024	A	G	2.9%	1415	2165	52	56	48.1%	51.9%	Synonymous mutation	UL46
CNS15	90025	A	T	2.9%	1385	2172	51	57	47.2%	52.8%	Non-synonymous mutation	UL46
CNS15	90026	G	C	2.9%	1422	2150	50	57	46.7%	53.3%	Non-synonymous mutation	UL46
CNS15	90027	A	T	2.9%	1385	2140	50	55	47.6%	52.4%	Synonymous mutation	UL46
CNS15	90028	C	T	2.9%	1408	2165	51	57	47.2%	52.8%	Non-synonymous mutation	UL46
CNS15	90037	G	A	2.8%	1375	2149	46	56	45.1%	54.9%	Non-synonymous mutation	UL46

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
CNS15	90179	C	T	3.5%	650	1698	23	62	27.1%	72.9%	Non-synonymous mutation	UL46
CNS15	92697	G	A	3.0%	1871	725	57	24	70.4%	29.6%	Synonymous mutation	UL47
CNS15	93057	T	C	2.9%	2888	1530	84	46	64.6%	35.4%	Synonymous mutation	UL47
CNS15	93752	T	C	2.1%	1571	1945	40	36	52.6%	47.4%	Non-synonymous mutation	UL47
CNS15	95589	C	T	2.5%	3157	2389	88	52	62.9%	37.1%	Synonymous mutation	UL48
CNS15	96380	T	C	4.4%	255	112	2	15	11.8%	88.2%	Intergenic	intergenic
CNS15	96970	C	T	2.6%	318	1215	14	27	34.1%	65.9%	Non-synonymous mutation	UL49
CNS15	96989	G	A	2.7%	369	1175	14	28	33.3%	66.7%	Synonymous mutation	UL49
CNS15	97258	C	G	3.3%	1618	793	67	15	81.7%	18.3%	Non-synonymous mutation	UL49
CNS15	97260	C	A	3.4%	1672	811	71	15	82.6%	17.4%	Non-synonymous mutation	UL49
CNS15	97319	G	A	3.0%	2065	1235	72	30	70.6%	29.4%	Synonymous mutation	UL49
CNS15	97469	G	C	2.4%	2220	1789	62	37	62.6%	37.4%	Intergenic	intergenic
CNS15	97675	A	C	2.3%	2794	1470	63	38	62.4%	37.6%	Intergenic	intergenic
CNS15	97698	G	A	2.9%	2661	1480	72	51	58.5%	41.5%	Intergenic	intergenic
CNS15	98239	G	T	2.1%	3070	1970	66	44	60.0%	40.0%	Synonymous mutation	UL50
CNS15	98942	C	G	2.2%	3133	2117	60	57	51.3%	48.7%	Non-synonymous mutation	UL50
CNS15	98947	A	C	2.2%	3031	2129	60	56	51.7%	48.3%	Synonymous mutation	UL50
CNS15	99140	C	T	2.6%	2030	1661	59	40	59.6%	40.4%	Intergenic	intergenic
CNS15	99538	A	G	2.5%	2351	2166	56	61	47.9%	52.1%	Synonymous mutation	UL51
CNS15	99547	A	G	2.6%	2294	2201	60	62	49.2%	50.8%	Synonymous mutation	UL51
CNS15	100664	G	A	2.5%	3068	1753	82	40	67.2%	32.8%	Non-synonymous mutation	UL52
CNS15	101138	A	G	2.6%	3093	2153	72	66	52.2%	47.8%	Non-synonymous mutation	UL52
CNS15	101173	C	T	2.4%	3065	2106	72	56	56.3%	43.8%	Synonymous mutation	UL52
CNS15	102640	T	C	2.7%	1034	2630	29	71	29.0%	71.0%	Synonymous mutation	UL52
CNS15	102667	A	G	2.9%	1485	2698	40	84	32.3%	67.7%	Synonymous mutation	UL52
CNS15	103586	G	A	2.3%	2044	1748	63	25	71.6%	28.4%	Non-synonymous mutation	UL53
CNS15	104069	G	C	2.2%	3118	815	71	16	81.6%	18.4%	Non-synonymous mutation	UL53
CNS15	104503	T	C	2.4%	2501	2763	79	52	60.3%	39.7%	Intergenic	intergenic
CNS15	104531	C	T	2.2%	2616	2982	72	54	57.1%	42.9%	Intergenic	intergenic
CNS15	104599	T	C	2.3%	2931	2546	64	62	50.8%	49.2%	Intergenic	intergenic
CNS15	104774	T	C	2.3%	2977	2572	72	58	55.4%	44.6%	Intergenic	intergenic
CNS15	107539	T	G	3.1%	679	1505	30	39	43.5%	56.5%	Non-synonymous mutation	UL56
CNS15	108306	C	T	2.2%	15	531	5	7	41.7%	58.3%	Intergenic	intergenic
CNS15	108617	G	A	12.0%	1069	225	149	28	84.2%	15.8%	Intergenic	intergenic
CNS15	108619	C	G	4.4%	1217	202	29	38	43.3%	56.7%	Intergenic	intergenic
CNS15	108621	C	G	8.3%	1099	247	107	15	87.7%	12.3%	Intergenic	intergenic
CNS15	108622	C	G	6.7%	1140	220	54	45	54.5%	45.5%	Intergenic	intergenic
CNS15	108623	A	G	9.1%	976	266	104	20	83.9%	16.1%	Intergenic	intergenic
CNS15	108624	C	G	14.3%	988	233	150	54	73.5%	26.5%	Intergenic	intergenic
CNS15	108625	A	G	7.0%	998	280	82	14	85.4%	14.6%	Intergenic	intergenic
CNS15	108626	C	A	4.9%	998	295	54	14	79.4%	20.6%	Intergenic	intergenic
CNS15	108627	C	G	5.9%	980	268	41	41	50.0%	50.0%	Intergenic	intergenic
CNS15	108629	A	G	5.9%	878	236	61	15	80.3%	19.7%	Intergenic	intergenic
CNS15	108634	A	C	5.8%	743	261	34	35	49.3%	50.7%	Intergenic	intergenic
CNS15	108636	C	G	2.4%	854	351	22	8	73.3%	26.7%	Intergenic	intergenic
CNS15	108644	G	C	7.3%	687	328	67	15	81.7%	18.3%	Intergenic	intergenic
CNS15	108750	C	T	2.8%	1144	431	38	8	82.6%	17.4%	Intergenic	intergenic
CNS15	108754	C	T	3.5%	1248	460	53	10	84.1%	15.9%	Intergenic	intergenic
CNS15	108756	C	T	2.4%	1350	518	32	14	69.6%	30.4%	Intergenic	intergenic
CNS15	108757	C	G	4.7%	1296	502	73	21	77.7%	22.3%	Intergenic	intergenic
CNS15	108758	C	G	8.1%	1265	480	123	47	72.4%	27.6%	Intergenic	intergenic
CNS15	108759	C	G	7.0%	1274	485	124	26	82.7%	17.3%	Intergenic	intergenic
CNS15	108760	C	G	2.6%	1579	562	37	19	66.1%	33.9%	Intergenic	intergenic
CNS15	108761	C	G	8.2%	1392	519	151	31	83.0%	17.0%	Intergenic	intergenic
CNS15	108768	A	G	25.2%	1237	439	440	130	77.2%	22.8%	Intergenic	intergenic
CNS15	108771	G	C	25.9%	1249	552	489	144	77.3%	22.7%	Intergenic	intergenic
CNS15	108773	C	G	23.7%	1353	605	472	146	76.4%	23.6%	Intergenic	intergenic
CNS15	108844	T	C	2.4%	1374	1018	20	38	34.5%	65.5%	Intergenic	intergenic
CNS15	109184	A	C	2.1%	35	1144	4	21	16.0%	84.0%	Intergenic	intergenic
CNS15	109216	A	G	3.4%	6	248	1	8	11.1%	88.9%	Intergenic	intergenic
CNS15	109690	C	T	2.9%	2859	1050	87	28	75.7%	24.3%	Intergenic	intergenic
CNS15	110582	C	A	2.5%	2621	540	65	17	79.3%	20.7%	Intergenic	intergenic
CNS15	110789	A	C	2.2%	1673	1314	19	49	27.9%	72.1%	Intergenic	intergenic
CNS15	110799	C	T	2.0%	1621	1751	37	33	52.9%	47.1%	Intergenic	intergenic
CNS15	110923	A	G	2.3%	1420	2633	36	59	37.9%	62.1%	Intergenic	intergenic
CNS15	112008	C	A	8.3%	230	165	23	13	63.9%	36.1%	Intergenic	intergenic
CNS15	112459	C	T	2.2%	956	2641	34	47	42.0%	58.0%	Intergenic	intergenic
CNS15	112533	A	G	2.8%	2343	3346	96	70	57.8%	42.2%	Intergenic	intergenic
CNS15	112953	G	A	3.3%	3312	3551	96	140	40.7%	59.3%	Non-synonymous mutation	RL2
CNS15	113197	A	C	2.2%	146	1841	8	36	18.2%	81.8%	Non-synonymous mutation	RL2
CNS15	113204	A	C	2.2%	122	1670	8	33	19.5%	80.5%	Synonymous mutation	RL2
CNS15	114875	G	A	2.8%	4964	3409	136	104	56.7%	43.3%	Synonymous mutation	RL2
CNS15	115404	C	T	3.5%	3405	2672	118	99	54.4%	45.6%	Intergenic	intergenic
CNS15	116580	A	G	7.1%	171	112	19	3	86.4%	13.6%	Non-synonymous mutation	RL1
CNS15	116630	G	A	5.8%	117	110	11	3	78.6%	21.4%	Synonymous mutation	RL1
CNS15	116639	C	T	10.1%	114	99	17	7	70.8%	29.2%	Synonymous mutation	RL1
CNS15	118653	T	C	3.9%	251	69	10	3	76.9%	23.1%	Intergenic	intergenic
CNS15	123366	G	A	3.3%	4059	2037	145	62	70.0%	30.0%	Intergenic	intergenic
CNS15	123506	G	A	3.6%	3820	2119	137	86	61.4%	38.6%	Intergenic	intergenic
CNS15	123544	C	T	11.4%	2538	3357	628	128	83.1%	16.9%	Intergenic	intergenic
CNS15	123790	C	T	37.0%	19	1086	82	569	12.6%	87.4%	Intergenic	intergenic
CNS15	124136	C	A	9.4%	664	12	52	18	74.3%	25.7%	Intergenic	intergenic
CNS15	124311	A	G	3.0%	533	589	20	15	57.1%	42.9%	Synonymous mutation	US1
CNS15	124401	A	G	3.5%	1212	1025	55	26	67.9%	32.1%	Synonymous mutation	US1
CNS15	124564	A	G	2.1%	1774	1329	30	37	44.8%	55.2%	Non-synonymous mutation	US1
CNS15	124584	A	C	2.2%	1865	1257	32	37	46.4%	53.6%	Synonymous mutation	US1
CNS15	124593	C	T	2.2%	1889	1264	30	40	42.9%	57.1%	Synonymous mutation	US1

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
CNS15	124647	T	C	2.4%	2070	1315	36	46	43.9%	56.1%	Synonymous mutation	US1
CNS15	124833	G	A	2.5%	2371	1520	73	28	72.3%	27.7%	Synonymous mutation	US1
CNS15	126141	G	T	2.9%	2074	1307	59	41	59.0%	41.0%	Non-synonymous mutation	US2
CNS15	126195	A	G	2.9%	2020	1340	53	46	53.5%	46.5%	Synonymous mutation	US2
CNS15	126393	G	C	2.4%	1916	1104	47	27	63.5%	36.5%	Synonymous mutation	US2
CNS15	126789	G	A	2.5%	1552	1078	37	29	56.1%	43.9%	Intergenic	intergenic
CNS15	127771	T	C	2.6%	2118	1473	60	36	62.5%	37.5%	Non-synonymous mutation	US3
CNS15	128076	G	A	2.6%	2613	2064	70	56	55.6%	44.4%	Synonymous mutation	US3
CNS15	128122	A	G	3.4%	2340	2147	79	81	49.4%	50.6%	Non-synonymous mutation	US3
CNS15	128157	G	A	3.6%	449	2334	18	87	17.1%	82.9%	Synonymous mutation	US3
CNS15	128822	C	G	3.3%	1877	2301	73	68	51.8%	48.2%	Synonymous mutation	US4
CNS15	129001	T	C	3.4%	2035	1495	65	60	52.0%	48.0%	Non-synonymous mutation	US4
CNS15	129281	G	A	2.9%	1947	1442	61	41	59.8%	40.2%	Synonymous mutation	US4
CNS15	129320	T	C	3.1%	1735	1472	54	47	53.5%	46.5%	Synonymous mutation	US4
CNS15	129403	G	T	3.6%	632	1652	29	55	34.5%	65.5%	Non-synonymous mutation	US4
CNS15	129658	C	T	3.5%	331	1320	15	45	25.0%	75.0%	Non-synonymous mutation	US4
CNS15	129691	A	G	3.2%	232	1039	10	32	23.8%	76.2%	Non-synonymous mutation	US4
CNS15	129711	T	C	2.9%	163	849	6	24	20.0%	80.0%	Non-synonymous mutation	US4
CNS15	129857	G	A	3.3%	234	469	12	12	50.0%	50.0%	Synonymous mutation	US4
CNS15	130109	G	T	3.3%	547	373	18	13	58.1%	41.9%	Non-synonymous mutation	US4
CNS15	130499	G	T	3.1%	803	929	32	23	58.2%	41.8%	Intergenic	intergenic
CNS15	130722	T	G	3.0%	1437	798	42	28	60.0%	40.0%	Intergenic	intergenic
CNS15	131193	C	T	3.0%	1515	860	53	20	72.6%	27.4%	Intergenic	intergenic
CNS15	131217	G	A	2.4%	1285	654	39	9	81.3%	18.8%	Intergenic	intergenic
CNS15	132252	A	C	3.2%	390	2314	54	36	60.0%	40.0%	Synonymous mutation	US6
CNS15	132575	C	T	3.4%	1435	1635	43	64	40.2%	59.8%	Non-synonymous mutation	US6
CNS15	132706	T	A	3.0%	1892	1597	53	53	50.0%	50.0%	Intergenic	intergenic
CNS15	132876	C	T	2.3%	2573	1428	56	39	58.9%	41.1%	Intergenic	intergenic
CNS15	133376	G	T	2.6%	1739	1845	58	36	61.7%	38.3%	Non-synonymous mutation	US7
CNS15	133518	A	G	4.5%	205	1280	20	50	28.6%	71.4%	Synonymous mutation	US7
CNS15	133543	C	T	4.8%	31	1170	8	53	13.1%	86.9%	Non-synonymous mutation	US7
CNS15	133659	C	T	2.1%	1196	554	21	17	55.3%	44.7%	Synonymous mutation	US7
CNS15	134778	G	A	3.0%	1987	1652	68	45	60.2%	39.8%	Non-synonymous mutation	US8
CNS15	135142	T	C	2.6%	2225	949	58	26	69.0%	31.0%	Non-synonymous mutation	US8
CNS15	135910	A	G	3.3%	2758	1817	83	72	53.5%	46.5%	Non-synonymous mutation	US8
CNS15	136398	G	A	3.7%	1585	2001	60	76	44.1%	55.9%	Synonymous mutation	US9
CNS15	136764	G	T	7.5%	918	976	53	100	34.6%	65.4%	Intergenic	intergenic
CNS15	136840	A	C	3.6%	1136	899	38	37	50.7%	49.3%	Intergenic	intergenic
CNS15	137554	G	C	2.5%	2213	1748	62	41	60.2%	39.8%	Synonymous mutation	US12
CNS15	137669	T	C	2.7%	1739	1506	49	41	54.4%	45.6%	Non-synonymous mutation	US12
CNS15	138511	G	A	2.4%	131	835	3	21	12.5%	87.5%	Synonymous mutation	US10
CNS17	11398	G	A	2.3%	140	951	4	22	15.4%	84.6%	Synonymous mutation	UL9
CNS17	18921	C	T	10.0%	801	985	98	101	49.2%	50.8%	Non-synonymous mutation	UL13
CNS17	21911	T	G	2.9%	708	125	8	17	32.0%	68.0%	Intergenic	intergenic
CNS17	29317	C	T	2.9%	1351	961	37	31	54.4%	45.6%	Non-synonymous mutation	UL19
CNS17	31693	G	A	2.5%	1273	941	28	29	49.1%	50.9%	Synonymous mutation	UL20
CNS17	39359	C	T	4.2%	287	453	26	6	81.3%	18.8%	Intergenic	intergenic
CNS17	62517	T	C	3.1%	19	262	8	1	88.9%	11.1%	Synonymous mutation	UL36
CNS17	64665	C	T	3.2%	1090	979	41	27	60.3%	39.7%	Synonymous mutation	UL36
CNS17	71472	C	A	9.9%	294	127	35	13	72.9%	27.1%	Intergenic	intergenic
CNS17	71473	A	C	4.8%	282	156	17	5	77.3%	22.7%	Intergenic	intergenic
CNS17	71511	C	T	2.0%	442	133	8	4	66.7%	33.3%	Intergenic	intergenic
CNS17	96188	A	G	5.2%	65	80	3	5	37.5%	62.5%	Intergenic	intergenic
CNS17	100673	G	A	2.4%	1108	776	28	19	59.6%	40.4%	Non-synonymous mutation	UL52
CNS17	108589	G	C	3.8%	971	90	13	30	30.2%	69.8%	Intergenic	intergenic
CNS17	108594	G	C	6.6%	1002	112	41	38	51.9%	48.1%	Intergenic	intergenic
CNS17	108595	G	A	11.2%	954	97	87	46	65.4%	34.6%	Intergenic	intergenic
CNS17	108598	C	G	5.2%	1020	152	48	16	75.0%	25.0%	Intergenic	intergenic
CNS17	108600	G	C	6.6%	984	138	51	32	61.4%	38.6%	Intergenic	intergenic
CNS17	108615	G	C	3.1%	947	240	34	4	89.5%	10.5%	Intergenic	intergenic
CNS17	108632	A	C	3.6%	680	277	31	5	86.1%	13.9%	Intergenic	intergenic
CNS17	108634	G	C	4.9%	671	285	43	6	87.8%	12.2%	Intergenic	intergenic
CNS17	108636	A	C	5.1%	644	282	43	7	86.0%	14.0%	Intergenic	intergenic
CNS17	108637	G	C	4.7%	656	286	38	8	82.6%	17.4%	Intergenic	intergenic
CNS17	108640	G	C	2.3%	643	289	19	3	86.4%	13.6%	Intergenic	intergenic
CNS17	108641	G	C	2.5%	646	287	21	3	87.5%	12.5%	Intergenic	intergenic
CNS17	117245	C	A	7.9%	184	13	15	2	88.2%	11.8%	Intergenic	intergenic
CNS17	117247	T	G	12.5%	143	9	19	3	86.4%	13.6%	Intergenic	intergenic
CNS17	117249	A	G	6.4%	136	8	9	1	90.0%	10.0%	Intergenic	intergenic
CNS17	124362	T	G	2.2%	2068	59	15	33	31.3%	68.8%	Intergenic	intergenic
CNS17	124365	T	C	2.8%	2064	60	19	43	30.6%	69.4%	Intergenic	intergenic
CNS17	124372	G	C	2.3%	2035	92	17	32	34.7%	65.3%	Intergenic	intergenic
CNS17	124373	C	G	2.3%	2028	60	18	31	36.7%	63.3%	Intergenic	intergenic
CNS17	125111	T	C	2.3%	592	58	12	3	80.0%	20.0%	Non-synonymous mutation	US1
CNS17	137578	G	T	2.2%	478	695	11	15	42.3%	57.7%	Intergenic	intergenic
SEM18	18144	A	C	3.6%	5100	3539	178	142	55.6%	44.4%	Non-synonymous mutation	UL13
SEM18	20222	G	T	3.3%	1948	4891	70	165	29.8%	70.2%	Non-synonymous mutation	UL15
SEM18	25427	C	T	4.0%	859	1634	14	89	13.6%	86.4%	Intergenic	intergenic
SEM18	26978	A	C	24.3%	1143	476	54	471	10.3%	89.7%	Intergenic	intergenic
SEM18	26979	A	C	28.3%	1135	487	88	572	13.3%	86.7%	Intergenic	intergenic
SEM18	26980	A	C	22.8%	1157	786	83	516	13.9%	86.1%	Intergenic	intergenic
SEM18	26981	A	C	8.2%	1205	1645	38	220	14.7%	85.3%	Intergenic	intergenic
SEM18	26982	A	C	2.5%	1254	2100	16	69	18.8%	81.2%	Intergenic	intergenic
SEM18	26983	A	C	3.1%	1223	1932	58	44	56.9%	43.1%	Intergenic	intergenic
SEM18	26984	A	C	8.1%	1193	1344	110	117	48.5%	51.5%	Intergenic	intergenic
SEM18	26985	A	G	4.6%	1224	1611	69	73	48.6%	51.4%	Intergenic	intergenic
SEM18	26986	A	G	9.8%	1242	1676	145	174	45.5%	54.5%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
SEM18	26987	A	G	4.2%	1485	2422	66	106	38.4%	61.6%	Intergenic	intergenic
SEM18	26995	A	G	3.8%	1141	3080	129	38	77.2%	22.8%	Intergenic	intergenic
SEM18	26996	A	G	2.3%	1112	3069	68	30	69.4%	30.6%	Intergenic	intergenic
SEM18	26997	A	G	3.1%	1024	3064	113	20	85.0%	15.0%	Intergenic	intergenic
SEM18	26998	A	G	6.3%	1020	3053	244	30	89.1%	10.9%	Intergenic	intergenic
SEM18	31312	G	A	4.9%	167	1307	10	66	13.2%	86.8%	Intergenic	intergenic
SEM18	31313	G	C	2.5%	164	1260	6	31	16.2%	83.8%	Intergenic	intergenic
SEM18	31315	A	G	76.1%	37	276	110	898	10.9%	89.1%	Intergenic	intergenic
SEM18	31318	A	G	21.6%	92	732	35	193	15.4%	84.6%	Intergenic	intergenic
SEM18	31319	A	G	53.7%	43	383	49	456	9.7%	90.3%	Intergenic	intergenic
SEM18	31320	C	G	98.4%	2	0	91	720	11.2%	88.8%	Intergenic	intergenic
SEM18	31321	C	G	37.9%	48	402	32	244	11.6%	88.4%	Intergenic	intergenic
SEM18	31322	C	G	62.3%	18	185	49	405	10.8%	89.2%	Intergenic	intergenic
SEM18	31326	G	A	42.0%	33	343	26	246	9.6%	90.4%	Intergenic	intergenic
SEM18	31335	G	T	18.9%	19	247	8	54	12.9%	87.1%	Intergenic	intergenic
SEM18	31380	C	G	10.9%	111	2	12	2	85.7%	14.3%	Intergenic	intergenic
SEM18	31397	C	G	3.7%	296	17	6	6	50.0%	50.0%	Intergenic	intergenic
SEM18	31398	T	G	18.6%	253	8	54	9	85.7%	14.3%	Intergenic	intergenic
SEM18	31410	G	C	66.4%	274	21	519	65	88.9%	11.1%	Intergenic	intergenic
SEM18	31412	G	C	67.4%	275	22	554	60	90.2%	9.8%	Intergenic	intergenic
SEM18	31415	T	G	68.3%	0	0	593	76	88.6%	11.4%	Intergenic	intergenic
SEM18	31425	C	A	99.9%	0	0	1047	112	90.3%	9.7%	Intergenic	intergenic
SEM18	31426	C	A	100.0%	0	0	1072	114	90.4%	9.6%	Intergenic	intergenic
SEM18	39512	T	C	2.2%	3330	2887	71	68	51.1%	48.9%	Intergenic	intergenic
SEM18	40087	G	C	2.3%	3481	2960	81	69	54.0%	46.0%	Synonymous mutation	UL25
SEM18	53561	T	G	2.6%	13863	8267	493	97	83.6%	16.4%	Intergenic	intergenic
SEM18	53563	C	G	3.2%	13879	8414	650	89	88.0%	12.0%	Intergenic	intergenic
SEM18	53577	T	G	2.5%	13828	7945	93	486	16.1%	83.9%	Intergenic	intergenic
SEM18	53578	G	C	2.2%	13788	8309	84	425	16.5%	83.5%	Intergenic	intergenic
SEM18	53579	A	G	2.5%	13419	8056	91	475	16.1%	83.9%	Intergenic	intergenic
SEM18	53587	C	G	2.2%	13036	8455	97	375	20.6%	79.4%	Intergenic	intergenic
SEM18	53588	T	G	2.0%	12450	8608	87	351	19.9%	80.1%	Intergenic	intergenic
SEM18	53591	C	G	2.3%	12629	8666	81	418	16.2%	83.8%	Intergenic	intergenic
SEM18	62870	G	T	3.3%	520	7	16	2	88.9%	11.1%	Synonymous mutation	UL36
SEM18	63669	A	G	2.1%	371	193	9	3	75.0%	25.0%	Non-synonymous mutation	UL36
SEM18	72170	A	C	3.2%	1872	1096	71	26	73.2%	26.8%	Intergenic	intergenic
SEM18	76018	C	T	2.0%	2379	2107	47	46	50.5%	49.5%	Intergenic	intergenic
SEM18	76021	C	G	2.2%	2345	2040	47	52	47.5%	52.5%	Intergenic	intergenic
SEM18	76025	A	T	5.9%	1424	743	67	71	48.6%	51.4%	Intergenic	intergenic
SEM18	76028	A	G	4.7%	1226	679	39	66	37.1%	62.9%	Intergenic	intergenic
SEM18	76030	C	T	5.4%	1521	752	97	37	72.4%	27.6%	Intergenic	intergenic
SEM18	76032	C	T	4.6%	1634	788	48	70	40.7%	59.3%	Intergenic	intergenic
SEM18	76033	C	T	13.7%	1457	692	233	118	66.4%	33.6%	Intergenic	intergenic
SEM18	76037	A	T	25.3%	956	566	365	204	64.1%	35.9%	Intergenic	intergenic
SEM18	76039	A	C	36.2%	899	530	425	387	52.3%	47.7%	Intergenic	intergenic
SEM18	76040	A	C	35.9%	983	514	454	389	53.9%	46.1%	Intergenic	intergenic
SEM18	76042	T	A	39.7%	727	406	490	315	60.9%	39.1%	Intergenic	intergenic
SEM18	76044	C	G	24.0%	1011	1859	482	429	52.9%	47.1%	Intergenic	intergenic
SEM18	76045	A	C	25.7%	1005	1867	693	318	68.5%	31.5%	Intergenic	intergenic
SEM18	76048	A	G	14.1%	1157	1966	368	151	70.9%	29.1%	Intergenic	intergenic
SEM18	76049	C	T	14.2%	1125	2050	304	228	57.1%	42.9%	Intergenic	intergenic
SEM18	76050	C	T	8.4%	1251	2226	219	100	68.7%	31.3%	Intergenic	intergenic
SEM18	76051	C	G	11.1%	1184	2123	205	209	49.5%	50.5%	Intergenic	intergenic
SEM18	76052	C	G	8.1%	1201	2133	154	139	52.6%	47.4%	Intergenic	intergenic
SEM18	76054	C	G	3.2%	1234	2153	73	39	65.2%	34.8%	Intergenic	intergenic
SEM18	77537	A	C	7.3%	878	353	40	57	41.2%	58.8%	Intergenic	intergenic
SEM18	77539	T	C	8.1%	634	324	30	55	35.3%	64.7%	Intergenic	intergenic
SEM18	77540	T	C	9.4%	617	233	43	54	44.3%	55.7%	Intergenic	intergenic
SEM18	80808	G	A	4.0%	3564	4458	141	197	41.7%	58.3%	Non-synonymous mutation	UL39
SEM18	97152	G	C	2.1%	79	445	9	2	81.8%	18.2%	Intergenic	intergenic
SEM18	109688	G	C	3.4%	8992	2737	116	299	28.0%	72.0%	Intergenic	intergenic
SEM18	109693	G	C	4.6%	9074	3083	217	367	37.2%	62.8%	Intergenic	intergenic
SEM18	109694	G	A	7.1%	8806	2824	414	485	46.1%	53.9%	Intergenic	intergenic
SEM18	109697	C	G	3.1%	9306	3479	196	213	47.9%	52.1%	Intergenic	intergenic
SEM18	109699	G	C	4.1%	8884	3283	221	315	41.2%	58.8%	Intergenic	intergenic
SEM18	110140	G	A	4.4%	55	485	16	9	64.0%	36.0%	Intergenic	intergenic
SEM18	112095	A	C	5.5%	1379	1087	16	127	11.2%	88.8%	Intergenic	intergenic
SEM18	117453	C	T	2.7%	245	189	8	4	66.7%	33.3%	Synonymous mutation	RL1
SEM18	117668	G	A	3.0%	217	33	6	2	75.0%	25.0%	Intergenic	intergenic
SEM18	119441	T	C	2.6%	19	388	8	3	72.7%	27.3%	Intergenic	intergenic
SEM18	123523	G	T	2.3%	109	1069	22	6	78.6%	21.4%	Intergenic	intergenic
SEM18	124582	C	T	23.7%	19	4020	154	1103	12.3%	87.7%	Intergenic	intergenic
SEM18	124709	C	A	4.7%	37	208	8	4	66.7%	33.3%	Intergenic	intergenic
SEM18	128558	C	T	7.7%	4354	2564	373	206	64.4%	35.6%	Synonymous mutation	US3
SEM18	139261	G	C	3.4%	64	649	4	38	9.5%	90.5%	Non-synonymous mutation	US10
DISS29	279	T	G	44.6%	460	12	300	80	78.9%	21.1%	Synonymous mutation	UL1
DISS29	518	T	C	32.0%	357	443	238	139	63.1%	36.9%	Non-synonymous mutation	UL1
DISS29	717	C	A	26.9%	550	352	241	90	72.8%	27.2%	Non-synonymous mutation	UL2
DISS29	747	T	A	17.6%	646	395	167	55	75.2%	24.8%	Non-synonymous mutation	UL2
DISS29	983	T	C	2.6%	667	507	19	12	61.3%	38.7%	Non-synonymous mutation	UL2
DISS29	1534	T	G	2.1%	667	340	13	9	59.1%	40.9%	Synonymous mutation	UL2
DISS29	1553	G	A	2.1%	653	298	13	7	65.0%	35.0%	Intergenic	intergenic
DISS29	2603	A	C	8.1%	607	239	53	22	70.7%	29.3%	Non-synonymous mutation	UL4
DISS29	2643	C	T	8.2%	639	226	56	21	72.7%	27.3%	Synonymous mutation	UL4
DISS29	2817	C	T	8.7%	665	395	84	17	83.2%	16.8%	Synonymous mutation	UL4
DISS29	2977	G	T	9.7%	757	344	85	33	72.0%	28.0%	Non-synonymous mutation	UL4
DISS29	3539	C	T	11.8%	297	596	64	55	53.8%	46.2%	Non-synonymous mutation	UL5

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	3591	C	T	9.5%	432	566	64	41	61.0%	39.0%	Synonymous mutation	UL5
DISS29	3611	T	C	12.3%	456	537	96	43	69.1%	30.9%	Non-synonymous mutation	UL5
DISS29	4047	C	T	31.3%	437	371	255	113	69.3%	30.7%	Synonymous mutation	UL5
DISS29	4398	A	G	20.0%	564	435	186	65	74.1%	25.9%	Synonymous mutation	UL5
DISS29	4545	A	G	11.8%	731	312	111	28	79.9%	20.1%	Synonymous mutation	UL5
DISS29	6313	C	T	22.0%	620	392	212	73	74.4%	25.6%	Synonymous mutation	UL6
DISS29	6322	C	T	2.0%	790	477	17	9	65.4%	34.6%	Synonymous mutation	UL6
DISS29	7009	G	C	13.9%	167	314	41	37	52.6%	47.4%	Non-synonymous mutation	UL6
DISS29	8005	A	G	30.6%	535	398	295	117	71.6%	28.4%	Synonymous mutation	UL7
DISS29	8635	T	A	11.7%	494	392	87	30	74.4%	25.6%	Non-synonymous mutation	UL7
DISS29	8807	T	C	10.7%	528	336	71	32	68.9%	31.1%	Intergenic	intergenic
DISS29	8931	C	T	9.4%	592	205	69	14	83.1%	16.9%	Intergenic	intergenic
DISS29	9366	C	T	16.9%	569	414	75	125	37.5%	62.5%	Non-synonymous mutation	UL8
DISS29	9438	T	C	34.4%	116	457	86	214	28.7%	71.3%	Non-synonymous mutation	UL8
DISS29	9450	C	T	8.0%	209	620	25	47	34.7%	65.3%	Non-synonymous mutation	UL8
DISS29	9817	T	C	28.9%	379	345	214	81	72.5%	27.5%	Synonymous mutation	UL8
DISS29	10459	G	A	8.9%	730	246	75	20	78.9%	21.1%	Synonymous mutation	UL8
DISS29	10537	G	T	21.5%	601	350	198	64	75.6%	24.4%	Synonymous mutation	UL8
DISS29	10978	C	A	9.6%	559	295	61	30	67.0%	33.0%	Synonymous mutation	UL8
DISS29	11077	G	A	12.3%	475	329	67	46	59.3%	40.7%	Synonymous mutation	UL8
DISS29	11122	A	G	27.6%	395	324	192	83	69.8%	30.2%	Synonymous mutation	UL8
DISS29	11492	T	C	33.5%	250	404	194	136	58.8%	41.2%	Synonymous mutation	UL9
DISS29	12281	C	G	22.0%	634	412	229	66	77.6%	22.4%	Synonymous mutation	UL9
DISS29	12413	G	A	20.4%	690	370	209	63	76.8%	23.2%	Synonymous mutation	UL9
DISS29	12501	C	T	30.1%	589	340	325	76	81.0%	19.0%	Non-synonymous mutation	UL9
DISS29	13028	G	C	8.6%	667	378	73	25	74.5%	25.5%	Synonymous mutation	UL9
DISS29	13033	G	C	30.1%	480	314	247	99	71.4%	28.6%	Non-synonymous mutation	UL9
DISS29	13823	C	G	30.4%	509	361	287	93	75.5%	24.5%	Synonymous mutation	UL9
DISS29	14261	T	C	33.0%	430	271	258	89	74.4%	25.6%	Synonymous mutation	UL10
DISS29	14513	C	G	30.7%	565	392	295	130	69.4%	30.6%	Synonymous mutation	UL10
DISS29	15284	A	C	13.9%	64	315	48	13	78.7%	21.3%	Intergenic	intergenic
DISS29	17285	G	C	8.8%	475	233	50	18	73.5%	26.5%	Non-synonymous mutation	UL12
DISS29	17318	T	C	40.3%	266	164	211	82	72.0%	28.0%	Non-synonymous mutation	UL12
DISS29	17427	C	T	10.7%	573	400	89	28	76.1%	23.9%	Non-synonymous mutation	UL12
DISS29	17648	C	T	45.8%	225	38	84	138	37.8%	62.2%	Synonymous mutation	UL13
DISS29	18227	G	A	20.6%	621	304	182	58	75.8%	24.2%	Synonymous mutation	UL13
DISS29	18449	A	G	33.5%	471	324	293	108	73.1%	26.9%	Synonymous mutation	UL13
DISS29	18506	T	C	9.1%	749	376	85	27	75.9%	24.1%	Synonymous mutation	UL13
DISS29	18641	T	C	8.8%	781	393	99	15	86.8%	13.2%	Synonymous mutation	UL13
DISS29	18746	C	T	2.4%	769	432	20	10	66.7%	33.3%	Synonymous mutation	UL13
DISS29	18829	A	G	11.9%	569	416	83	50	62.4%	37.6%	Non-synonymous mutation	UL13
DISS29	19312	G	T	8.7%	788	408	84	30	73.7%	26.3%	Synonymous mutation	UL14
DISS29	20396	C	A	20.5%	497	367	158	65	70.9%	29.1%	Synonymous mutation	UL15
DISS29	20677	A	C	2.3%	660	488	4	23	14.8%	85.2%	Intergenic	intergenic
DISS29	20843	C	T	20.7%	474	389	139	87	61.5%	38.5%	Non-synonymous mutation	UL16
DISS29	21223	G	A	22.7%	514	357	177	79	69.1%	30.9%	Synonymous mutation	UL16
DISS29	21354	C	T	10.4%	665	322	93	22	80.9%	19.1%	Non-synonymous mutation	UL16
DISS29	21952	C	T	12.2%	488	287	82	26	75.9%	24.1%	Non-synonymous mutation	UL17
DISS29	21953	C	T	12.0%	489	292	82	24	77.4%	22.6%	Synonymous mutation	UL17
DISS29	22145	C	T	33.3%	289	355	205	117	63.7%	36.3%	Synonymous mutation	UL17
DISS29	22636	T	C	8.6%	661	461	79	27	74.5%	25.5%	Non-synonymous mutation	UL17
DISS29	22815	C	A	23.4%	405	429	163	92	63.9%	36.1%	Non-synonymous mutation	UL17
DISS29	22816	G	C	23.3%	404	424	159	92	63.3%	36.7%	Non-synonymous mutation	UL17
DISS29	22817	T	G	23.4%	386	427	158	90	63.7%	36.3%	Synonymous mutation	UL17
DISS29	23065	C	T	39.3%	59	204	86	84	50.6%	49.4%	Non-synonymous mutation	UL17
DISS29	23845	C	T	19.2%	533	423	157	70	69.2%	30.8%	Non-synonymous mutation	UL17
DISS29	25385	T	C	14.4%	82	108	17	15	53.1%	46.9%	Non-synonymous mutation	UL15
DISS29	25428	C	A	11.1%	70	113	8	15	34.8%	65.2%	Synonymous mutation	UL15
DISS29	26524	T	C	5.9%	334	129	21	8	72.4%	27.6%	Intergenic	intergenic
DISS29	26567	C	A	3.9%	346	122	12	7	63.2%	36.8%	Intergenic	intergenic
DISS29	26704	G	T	36.1%	352	40	175	46	79.2%	20.8%	Intergenic	intergenic
DISS29	28031	T	C	6.6%	381	332	37	15	71.2%	28.8%	Intergenic	intergenic
DISS29	28032	G	C	32.5%	417	357	295	78	79.1%	20.9%	Intergenic	intergenic
DISS29	28576	C	T	2.3%	745	419	22	6	78.6%	21.4%	Synonymous mutation	UL19
DISS29	29012	T	C	30.5%	504	376	297	90	76.7%	23.3%	Non-synonymous mutation	UL19
DISS29	29731	G	A	26.4%	565	384	243	98	71.3%	28.7%	Synonymous mutation	UL19
DISS29	30001	C	T	9.5%	707	421	85	33	72.0%	28.0%	Synonymous mutation	UL19
DISS29	31043	A	G	21.4%	457	453	152	96	61.3%	38.7%	Non-synonymous mutation	UL19
DISS29	31948	G	T	2.5%	596	531	18	11	62.1%	37.9%	Synonymous mutation	UL19
DISS29	32059	G	C	22.3%	475	435	178	84	67.9%	32.1%	Synonymous mutation	UL19
DISS29	32703	T	C	35.8%	415	168	243	85	74.1%	25.9%	Non-synonymous mutation	UL20
DISS29	32876	A	G	33.8%	335	375	213	150	58.7%	41.3%	Non-synonymous mutation	UL20
DISS29	33192	C	T	24.7%	261	433	126	102	55.3%	44.7%	Non-synonymous mutation	UL20
DISS29	33578	G	A	25.2%	429	351	171	92	65.0%	35.0%	Intergenic	intergenic
DISS29	33613	A	C	12.6%	281	425	80	22	78.4%	21.6%	Intergenic	intergenic
DISS29	33622	T	G	14.3%	282	406	83	32	72.2%	27.8%	Intergenic	intergenic
DISS29	34718	A	C	37.9%	389	306	269	157	63.1%	36.9%	Non-synonymous mutation	UL21
DISS29	34719	C	T	24.0%	477	388	186	87	68.1%	31.9%	Non-synonymous mutation	UL21
DISS29	35576	A	G	2.5%	579	35	3	13	18.8%	81.3%	Intergenic	intergenic
DISS29	35769	C	G	3.0%	431	451	14	13	51.9%	48.1%	Synonymous mutation	UL22
DISS29	36358	A	G	33.0%	458	397	317	108	74.6%	25.4%	Non-synonymous mutation	UL22
DISS29	36546	C	A	32.8%	503	308	286	109	72.4%	27.6%	Synonymous mutation	UL22
DISS29	37416	C	G	2.9%	264	553	9	15	37.5%	62.5%	Synonymous mutation	UL22
DISS29	37443	C	T	3.0%	359	562	13	16	44.8%	55.2%	Synonymous mutation	UL22
DISS29	37596	T	C	10.6%	647	445	103	27	79.2%	20.8%	Synonymous mutation	UL22
DISS29	37644	T	C	13.7%	699	409	131	45	74.4%	25.6%	Synonymous mutation	UL22
DISS29	37646	T	C	13.1%	697	386	125	41	75.3%	24.7%	Non-synonymous mutation	UL22

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	37815	T	G	19.8%	154	549	70	103	40.5%	59.5%	Synonymous mutation	UL22
DISS29	37912	G	C	10.2%	387	578	59	51	53.6%	46.4%	Non-synonymous mutation	UL22
DISS29	39486	T	C	30.9%	515	280	268	89	75.1%	24.9%	Non-synonymous mutation	UL23
DISS29	39511	C	T	20.2%	598	332	177	59	75.0%	25.0%	Synonymous mutation	UL23
DISS29	39780	A	C	29.5%	481	394	254	113	69.2%	30.8%	Synonymous mutation	UL24
DISS29	39896	T	C	28.3%	521	382	274	82	77.0%	23.0%	Non-synonymous mutation	UL24
DISS29	39900	T	C	28.9%	520	380	286	81	77.9%	22.1%	Synonymous mutation	UL24
DISS29	40371	C	T	19.8%	300	333	86	70	55.1%	44.9%	Synonymous mutation	UL24
DISS29	40489	C	T	6.6%	254	211	26	7	78.8%	21.2%	Intergenic	intergenic
DISS29	40490	C	T	5.9%	250	212	23	6	79.3%	20.7%	Intergenic	intergenic
DISS29	40492	C	T	5.1%	255	212	21	4	84.0%	16.0%	Intergenic	intergenic
DISS29	40493	G	T	5.0%	251	205	21	3	87.5%	12.5%	Intergenic	intergenic
DISS29	40512	C	T	11.3%	70	271	37	7	84.1%	15.9%	Intergenic	intergenic
DISS29	40521	T	C	2.6%	60	232	3	5	37.5%	62.5%	Intergenic	intergenic
DISS29	40666	T	C	35.7%	317	214	264	38	87.4%	12.6%	Intergenic	intergenic
DISS29	42540	T	C	8.9%	588	410	70	28	71.4%	28.6%	Intergenic	intergenic
DISS29	42714	T	G	9.6%	555	438	79	27	74.5%	25.5%	Intergenic	intergenic
DISS29	43631	T	G	5.5%	481	364	40	9	81.6%	18.4%	Synonymous mutation	UL26
DISS29	43748	T	C	2.4%	788	377	23	5	82.1%	17.9%	Synonymous mutation	UL26
DISS29	43820	G	T	8.3%	679	406	77	22	77.8%	22.2%	Synonymous mutation	UL26
DISS29	44417	C	A	11.0%	3	199	4	21	16.0%	84.0%	Synonymous mutation	UL26
DISS29	44825	T	G	5.3%	117	8	1	6	14.3%	85.7%	Intergenic	intergenic
DISS29	44925	G	T	2.4%	301	142	7	4	63.6%	36.4%	Intergenic	intergenic
DISS29	44926	A	T	4.5%	299	141	12	9	57.1%	42.9%	Intergenic	intergenic
DISS29	44951	C	G	2.0%	362	123	4	6	40.0%	60.0%	Intergenic	intergenic
DISS29	44952	C	G	2.2%	367	122	5	6	45.5%	54.5%	Intergenic	intergenic
DISS29	44989	C	T	39.6%	255	70	167	47	78.0%	22.0%	Intergenic	intergenic
DISS29	45000	C	A	2.8%	223	119	7	3	70.0%	30.0%	Intergenic	intergenic
DISS29	45001	A	C	4.2%	205	115	5	9	35.7%	64.3%	Intergenic	intergenic
DISS29	45095	T	C	43.9%	109	174	129	94	57.8%	42.2%	Intergenic	intergenic
DISS29	45280	C	G	25.4%	494	261	198	60	76.7%	23.3%	Non-synonymous mutation	UL27
DISS29	45484	C	T	23.6%	485	352	169	90	65.3%	34.7%	Non-synonymous mutation	UL27
DISS29	45669	T	G	8.1%	659	490	62	40	60.8%	39.2%	Non-synonymous mutation	UL27
DISS29	46627	G	C	2.6%	816	487	21	14	60.0%	40.0%	Non-synonymous mutation	UL27
DISS29	47602	C	T	2.9%	155	345	6	9	40.0%	60.0%	Non-synonymous mutation	UL27
DISS29	47634	C	T	2.9%	105	259	6	5	54.5%	45.5%	Non-synonymous mutation	UL27
DISS29	47696	G	C	32.4%	26	74	22	26	45.8%	54.2%	Synonymous mutation	UL27
DISS29	48519	G	A	2.8%	326	393	13	8	61.9%	38.1%	Synonymous mutation	UL28
DISS29	48777	T	C	40.9%	117	34	94	12	88.7%	11.3%	Synonymous mutation	UL28
DISS29	49062	A	G	3.5%	149	343	6	12	33.3%	66.7%	Synonymous mutation	UL28
DISS29	49842	A	T	34.3%	507	326	321	114	73.8%	26.2%	Synonymous mutation	UL28
DISS29	50428	G	A	3.6%	121	252	7	7	50.0%	50.0%	Intergenic	intergenic
DISS29	51299	G	T	8.7%	658	334	63	31	67.0%	33.0%	Synonymous mutation	UL29
DISS29	51776	C	T	7.9%	442	534	56	28	66.7%	33.3%	Synonymous mutation	UL29
DISS29	51839	A	C	32.5%	351	423	265	109	70.9%	29.1%	Synonymous mutation	UL29
DISS29	52384	C	T	12.1%	685	439	115	40	74.2%	25.8%	Non-synonymous mutation	UL29
DISS29	52439	C	T	21.2%	115	510	50	118	29.8%	70.2%	Synonymous mutation	UL29
DISS29	53032	C	T	23.3%	549	376	187	95	66.3%	33.7%	Non-synonymous mutation	UL29
DISS29	53108	G	A	21.7%	532	393	177	80	68.9%	31.1%	Synonymous mutation	UL29
DISS29	53603	G	A	21.7%	581	348	205	53	79.5%	20.5%	Synonymous mutation	UL29
DISS29	53693	T	C	31.0%	538	309	305	76	80.1%	19.9%	Synonymous mutation	UL29
DISS29	53726	T	C	31.3%	487	347	296	85	77.7%	22.3%	Synonymous mutation	UL29
DISS29	53758	A	C	22.1%	535	340	189	60	75.9%	24.1%	Non-synonymous mutation	UL29
DISS29	53852	G	A	21.5%	482	367	156	78	66.7%	33.3%	Synonymous mutation	UL29
DISS29	54244	G	A	19.3%	651	285	174	50	77.7%	22.3%	Intergenic	intergenic
DISS29	54251	G	C	19.6%	648	284	179	48	78.9%	21.1%	Intergenic	intergenic
DISS29	54255	C	T	19.6%	645	283	181	46	79.7%	20.3%	Intergenic	intergenic
DISS29	54289	A	G	18.5%	761	299	208	32	86.7%	13.3%	Intergenic	intergenic
DISS29	54343	A	T	19.4%	817	347	210	70	75.0%	25.0%	Intergenic	intergenic
DISS29	54360	C	G	21.1%	855	388	224	110	67.1%	32.9%	Intergenic	intergenic
DISS29	54361	C	G	21.4%	862	381	226	113	66.7%	33.3%	Intergenic	intergenic
DISS29	54498	T	C	15.5%	944	609	213	72	74.7%	25.3%	Intergenic	intergenic
DISS29	54503	C	A	16.8%	936	620	244	72	77.2%	22.8%	Intergenic	intergenic
DISS29	54521	T	G	3.6%	1179	626	9	59	13.2%	86.8%	Intergenic	intergenic
DISS29	54523	T	C	4.3%	1169	614	15	67	18.3%	81.7%	Intergenic	intergenic
DISS29	54524	G	A	3.1%	1175	650	8	51	13.6%	86.4%	Intergenic	intergenic
DISS29	54525	A	T	3.7%	1148	622	10	59	14.5%	85.5%	Intergenic	intergenic
DISS29	54528	T	G	3.1%	1147	677	6	52	10.3%	89.7%	Intergenic	intergenic
DISS29	54542	A	G	18.6%	828	605	228	100	69.5%	30.5%	Intergenic	intergenic
DISS29	54617	T	C	13.7%	140	598	51	66	43.6%	56.4%	Intergenic	intergenic
DISS29	54652	C	G	26.4%	155	457	128	91	58.4%	41.6%	Intergenic	intergenic
DISS29	54655	C	G	25.3%	164	451	125	84	59.8%	40.2%	Intergenic	intergenic
DISS29	54707	G	A	67.8%	165	53	219	240	47.7%	52.3%	Intergenic	intergenic
DISS29	54710	C	T	19.3%	289	250	94	35	72.9%	27.1%	Intergenic	intergenic
DISS29	54722	C	G	3.6%	397	247	16	8	66.7%	33.3%	Intergenic	intergenic
DISS29	54729	G	T	4.6%	397	267	24	8	75.0%	25.0%	Intergenic	intergenic
DISS29	54731	G	A	5.5%	395	259	27	11	71.1%	28.9%	Intergenic	intergenic
DISS29	54734	C	G	38.1%	197	200	206	66	75.7%	24.3%	Intergenic	intergenic
DISS29	54735	T	C	39.3%	197	192	205	54	79.2%	20.8%	Intergenic	intergenic
DISS29	54753	A	G	3.1%	417	317	7	17	29.2%	70.8%	Intergenic	intergenic
DISS29	54759	A	C	29.9%	292	286	161	88	64.7%	35.3%	Intergenic	intergenic
DISS29	54765	T	C	11.0%	283	369	34	47	42.0%	58.0%	Intergenic	intergenic
DISS29	54766	G	C	23.5%	317	407	149	75	66.5%	33.5%	Intergenic	intergenic
DISS29	54768	C	T	23.1%	322	414	145	76	65.6%	34.4%	Intergenic	intergenic
DISS29	54769	G	A	23.5%	288	388	153	76	66.8%	33.2%	Intergenic	intergenic
DISS29	54786	A	G	30.8%	311	404	218	100	68.6%	31.4%	Intergenic	intergenic
DISS29	54791	T	C	30.9%	314	405	223	99	69.3%	30.7%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	54902	C	T	2.0%	650	411	17	5	77.3%	22.7%	Non-synonymous mutation	UL30
DISS29	54949	G	A	32.0%	436	328	252	108	70.0%	30.0%	Non-synonymous mutation	UL30
DISS29	55207	G	A	2.9%	683	484	27	8	77.1%	22.9%	Non-synonymous mutation	UL30
DISS29	55222	C	A	32.4%	435	386	276	118	70.1%	29.9%	Non-synonymous mutation	UL30
DISS29	55452	G	T	31.3%	468	239	263	61	81.2%	18.8%	Synonymous mutation	UL30
DISS29	56466	T	C	35.3%	320	366	239	136	63.7%	36.3%	Synonymous mutation	UL30
DISS29	56728	A	G	37.7%	376	245	288	88	76.6%	23.4%	Non-synonymous mutation	UL30
DISS29	56744	C	A	3.9%	38	281	7	6	53.8%	46.2%	Non-synonymous mutation	UL30
DISS29	56795	G	A	45.9%	100	319	247	112	68.8%	31.2%	Non-synonymous mutation	UL30
DISS29	56816	G	A	44.2%	136	290	227	111	67.2%	32.8%	Non-synonymous mutation	UL30
DISS29	56829	G	C	3.7%	201	292	11	8	57.9%	42.1%	Non-synonymous mutation	UL30
DISS29	56831	C	A	14.6%	178	265	41	35	53.9%	46.1%	Non-synonymous mutation	UL30
DISS29	56834	A	G	5.1%	384	357	21	19	52.5%	47.5%	Non-synonymous mutation	UL30
DISS29	56840	G	A	3.3%	408	349	14	12	53.8%	46.2%	Non-synonymous mutation	UL30
DISS29	56843	C	A	42.6%	199	245	226	105	68.3%	31.7%	Non-synonymous mutation	UL30
DISS29	56846	A	G	2.0%	426	344	11	5	68.8%	31.3%	Non-synonymous mutation	UL30
DISS29	57306	T	C	32.5%	490	283	291	82	78.0%	22.0%	Synonymous mutation	UL30
DISS29	57853	A	G	35.3%	222	418	187	162	53.6%	46.4%	Non-synonymous mutation	UL30
DISS29	58440	A	C	35.2%	452	374	322	126	71.9%	28.1%	Synonymous mutation	UL30
DISS29	59144	G	T	35.1%	427	234	271	87	75.7%	24.3%	Synonymous mutation	UL31
DISS29	59670	C	G	32.0%	415	358	255	111	69.7%	30.3%	Synonymous mutation	UL32
DISS29	60358	G	C	4.5%	504	324	19	20	48.7%	51.3%	Non-synonymous mutation	UL32
DISS29	60360	C	G	4.7%	506	339	23	19	54.8%	45.2%	Synonymous mutation	UL32
DISS29	60567	G	A	26.5%	413	443	217	92	70.2%	29.8%	Synonymous mutation	UL32
DISS29	60852	G	C	29.7%	60	189	52	53	49.5%	50.5%	Synonymous mutation	UL32
DISS29	60912	C	G	16.5%	107	90	27	12	69.2%	30.8%	Synonymous mutation	UL32
DISS29	61168	A	G	36.9%	459	104	255	75	77.3%	22.7%	Non-synonymous mutation	UL32
DISS29	61182	G	T	35.4%	457	131	242	80	75.2%	24.8%	Synonymous mutation	UL33
DISS29	61642	C	A	9.1%	632	271	81	10	89.0%	11.0%	Intergenic	intergenic
DISS29	61740	G	A	23.7%	478	368	175	88	66.5%	33.5%	Non-synonymous mutation	UL34
DISS29	61949	T	G	29.8%	387	365	221	98	69.3%	30.7%	Synonymous mutation	UL34
DISS29	62123	C	T	32.4%	271	345	180	115	61.0%	39.0%	Synonymous mutation	UL34
DISS29	62336	A	G	8.0%	528	358	62	15	80.5%	19.5%	Synonymous mutation	UL34
DISS29	62979	T	C	24.0%	311	305	93	102	47.7%	52.3%	Intergenic	intergenic
DISS29	62982	C	T	23.1%	314	307	94	93	50.3%	49.7%	Intergenic	intergenic
DISS29	63422	G	A	12.8%	441	417	81	45	64.3%	35.7%	Non-synonymous mutation	UL36
DISS29	63516	G	C	11.1%	400	354	68	26	72.3%	27.7%	Synonymous mutation	UL36
DISS29	63620	T	G	2.2%	304	186	8	3	72.7%	27.3%	Non-synonymous mutation	UL36
DISS29	63626	T	G	2.7%	285	180	10	3	76.9%	23.1%	Non-synonymous mutation	UL36
DISS29	63629	C	G	2.8%	283	168	11	2	84.6%	15.4%	Non-synonymous mutation	UL36
DISS29	63674	C	G	5.0%	265	95	12	7	63.2%	36.8%	Non-synonymous mutation	UL36
DISS29	63675	C	G	5.0%	251	93	11	8	57.9%	42.1%	Synonymous mutation	UL36
DISS29	63677	A	G	3.6%	257	88	8	5	61.5%	38.5%	Non-synonymous mutation	UL36
DISS29	63842	G	T	42.5%	130	149	176	30	85.4%	14.6%	Non-synonymous mutation	UL36
DISS29	64069	G	A	2.7%	237	44	6	2	75.0%	25.0%	Non-synonymous mutation	UL36
DISS29	64429	G	A	29.2%	412	168	180	60	75.0%	25.0%	Non-synonymous mutation	UL36
DISS29	64527	A	G	38.3%	309	178	251	53	82.6%	17.4%	Synonymous mutation	UL36
DISS29	64571	T	G	3.5%	308	250	5	15	25.0%	75.0%	Non-synonymous mutation	UL36
DISS29	64581	A	G	32.1%	230	181	86	109	44.1%	55.9%	Synonymous mutation	UL36
DISS29	64972	C	A	32.8%	450	225	242	88	73.3%	26.7%	Non-synonymous mutation	UL36
DISS29	65838	C	T	22.6%	531	389	178	91	66.2%	33.8%	Synonymous mutation	UL36
DISS29	65900	C	T	21.7%	527	409	175	85	67.3%	32.7%	Non-synonymous mutation	UL36
DISS29	67215	A	G	36.3%	421	375	283	172	62.2%	37.8%	Synonymous mutation	UL36
DISS29	67358	T	C	34.4%	480	295	304	103	74.7%	25.3%	Non-synonymous mutation	UL36
DISS29	67490	C	G	2.2%	713	372	20	4	83.3%	16.7%	Non-synonymous mutation	UL36
DISS29	67809	G	A	22.2%	623	434	207	96	68.3%	31.7%	Synonymous mutation	UL36
DISS29	68761	G	A	19.4%	271	304	76	62	55.1%	44.9%	Non-synonymous mutation	UL36
DISS29	68797	A	G	31.8%	224	256	133	91	59.4%	40.6%	Non-synonymous mutation	UL36
DISS29	69142	C	G	9.0%	633	303	67	25	72.8%	27.2%	Non-synonymous mutation	UL36
DISS29	69417	T	C	32.0%	486	318	266	113	70.2%	29.8%	Synonymous mutation	UL36
DISS29	70002	C	T	9.5%	343	293	44	23	65.7%	34.3%	Synonymous mutation	UL36
DISS29	70356	G	A	23.1%	577	237	186	59	75.9%	24.1%	Synonymous mutation	UL36
DISS29	70932	A	G	32.4%	303	297	191	100	65.6%	34.4%	Synonymous mutation	UL36
DISS29	71040	C	T	36.2%	300	297	243	97	71.5%	28.5%	Synonymous mutation	UL36
DISS29	71447	C	G	35.3%	423	304	250	146	63.1%	36.9%	Non-synonymous mutation	UL36
DISS29	72569	A	C	20.7%	80	66	18	21	46.2%	53.8%	Intergenic	intergenic
DISS29	72570	A	C	7.8%	100	88	7	9	43.8%	56.3%	Intergenic	intergenic
DISS29	72648	C	A	15.7%	290	169	35	51	40.7%	59.3%	Non-synonymous mutation	UL37
DISS29	72733	A	G	34.6%	385	127	212	59	78.2%	21.8%	Non-synonymous mutation	UL37
DISS29	75330	C	T	23.2%	482	285	178	53	77.1%	22.9%	Synonymous mutation	UL37
DISS29	75393	G	C	32.6%	453	219	261	65	80.1%	19.9%	Non-synonymous mutation	UL37
DISS29	75927	C	T	9.5%	569	410	65	38	63.1%	36.9%	Synonymous mutation	UL37
DISS29	76215	T	G	3.2%	206	63	8	1	88.9%	11.1%	Intergenic	intergenic
DISS29	76219	T	G	4.5%	149	51	6	4	60.0%	40.0%	Intergenic	intergenic
DISS29	76221	T	G	15.8%	139	46	22	13	62.9%	37.1%	Intergenic	intergenic
DISS29	76223	T	G	12.3%	139	54	14	15	48.3%	51.7%	Intergenic	intergenic
DISS29	76225	T	G	26.3%	136	53	26	43	37.7%	62.3%	Intergenic	intergenic
DISS29	76227	T	G	50.0%	73	57	117	68	63.2%	36.8%	Intergenic	intergenic
DISS29	76240	C	G	11.6%	186	347	30	40	42.9%	57.1%	Intergenic	intergenic
DISS29	76288	C	T	11.7%	250	447	47	45	51.1%	48.9%	Intergenic	intergenic
DISS29	76378	G	T	11.4%	452	319	74	25	74.7%	25.3%	Intergenic	intergenic
DISS29	76436	A	C	3.8%	497	250	14	16	46.7%	53.3%	Intergenic	intergenic
DISS29	76442	A	C	8.7%	457	340	63	13	82.9%	17.1%	Intergenic	intergenic
DISS29	76445	A	C	23.5%	428	252	102	109	48.3%	51.7%	Intergenic	intergenic
DISS29	76452	A	C	13.0%	412	350	78	36	68.4%	31.6%	Intergenic	intergenic
DISS29	76454	A	C	13.1%	407	359	80	36	69.0%	31.0%	Intergenic	intergenic
DISS29	76551	A	G	14.1%	463	226	61	52	54.0%	46.0%	Non-synonymous mutation	UL38

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	76667	C	G	49.0%	376	15	260	120	68.4%	31.6%	Synonymous mutation	UL38
DISS29	77067	G	A	23.2%	525	359	183	85	68.3%	31.7%	Non-synonymous mutation	UL38
DISS29	77590	G	A	22.6%	472	394	191	62	75.5%	24.5%	Non-synonymous mutation	UL38
DISS29	77936	T	C	16.7%	221	139	43	39	52.4%	47.6%	Intergenic	intergenic
DISS29	77954	A	C	2.4%	63	217	6	1	85.7%	14.3%	Intergenic	intergenic
DISS29	78364	T	C	30.5%	514	290	263	91	74.3%	25.7%	Intergenic	intergenic
DISS29	78684	T	C	36.3%	89	394	97	179	35.1%	64.9%	Synonymous mutation	UL39
DISS29	78758	C	G	9.8%	349	454	53	34	60.9%	39.1%	Non-synonymous mutation	UL39
DISS29	78924	T	C	32.9%	184	343	111	147	43.0%	57.0%	Synonymous mutation	UL39
DISS29	79338	T	C	30.0%	380	357	225	91	71.2%	28.8%	Synonymous mutation	UL39
DISS29	79378	A	G	19.7%	461	389	153	56	73.2%	26.8%	Non-synonymous mutation	UL39
DISS29	79535	G	A	30.3%	460	249	242	66	78.6%	21.4%	Non-synonymous mutation	UL39
DISS29	79759	G	A	9.7%	698	439	86	36	70.5%	29.5%	Non-synonymous mutation	UL39
DISS29	79767	T	C	20.4%	609	397	184	74	71.3%	28.7%	Synonymous mutation	UL39
DISS29	80052	C	T	31.6%	339	406	231	113	67.2%	32.8%	Synonymous mutation	UL39
DISS29	80630	G	C	30.3%	473	299	247	89	73.5%	26.5%	Non-synonymous mutation	UL39
DISS29	80835	T	C	29.1%	408	315	196	100	66.2%	33.8%	Synonymous mutation	UL39
DISS29	80856	G	C	27.5%	422	305	184	91	66.9%	33.1%	Synonymous mutation	UL39
DISS29	80872	T	G	25.7%	426	283	165	80	67.3%	32.7%	Non-synonymous mutation	UL39
DISS29	80876	G	C	25.7%	429	280	164	81	66.9%	33.1%	Non-synonymous mutation	UL39
DISS29	80880	A	G	25.8%	417	275	163	77	67.9%	32.1%	Synonymous mutation	UL39
DISS29	80883	C	A	25.3%	433	268	164	73	69.2%	30.8%	Synonymous mutation	UL39
DISS29	80889	C	T	24.4%	438	269	157	71	68.9%	31.1%	Synonymous mutation	UL39
DISS29	80901	C	T	15.1%	499	281	98	41	70.5%	29.5%	Synonymous mutation	UL39
DISS29	80907	C	A	24.2%	445	250	154	68	69.4%	30.6%	Synonymous mutation	UL39
DISS29	80935	C	A	22.9%	468	201	147	52	73.9%	26.1%	Synonymous mutation	UL39
DISS29	80939	G	A	22.9%	477	187	147	51	74.2%	25.8%	Non-synonymous mutation	UL39
DISS29	80946	C	T	22.8%	482	177	145	50	74.4%	25.6%	Synonymous mutation	UL39
DISS29	80949	T	C	22.6%	474	177	140	50	73.7%	26.3%	Synonymous mutation	UL39
DISS29	80955	C	G	22.2%	476	169	135	49	73.4%	26.6%	Synonymous mutation	UL39
DISS29	80956	A	C	22.6%	474	165	137	50	73.3%	26.7%	Non-synonymous mutation	UL39
DISS29	80957	T	G	22.6%	478	163	138	49	73.8%	26.2%	Non-synonymous mutation	UL39
DISS29	80988	A	G	23.3%	453	64	128	29	81.5%	18.5%	Synonymous mutation	UL39
DISS29	80994	T	G	22.5%	451	45	119	25	82.6%	17.4%	Synonymous mutation	UL39
DISS29	80997	T	C	22.3%	447	30	117	20	85.4%	14.6%	Synonymous mutation	UL39
DISS29	81006	A	C	25.9%	54	360	50	95	34.5%	65.5%	Synonymous mutation	UL39
DISS29	81018	G	A	23.7%	100	384	52	98	34.7%	65.3%	Synonymous mutation	UL39
DISS29	81021	G	A	23.2%	107	393	51	100	33.8%	66.2%	Synonymous mutation	UL39
DISS29	81024	G	C	22.7%	117	393	49	101	32.7%	67.3%	Synonymous mutation	UL39
DISS29	81037	G	A	22.8%	137	420	54	110	32.9%	67.1%	Non-synonymous mutation	UL39
DISS29	81046	C	A	22.2%	160	407	51	111	31.5%	68.5%	Non-synonymous mutation	UL39
DISS29	81069	C	A	20.4%	210	412	60	99	37.7%	62.3%	Synonymous mutation	UL39
DISS29	81072	T	C	20.3%	211	415	61	98	38.4%	61.6%	Synonymous mutation	UL39
DISS29	81096	G	C	20.4%	239	409	79	87	47.6%	52.4%	Synonymous mutation	UL39
DISS29	81106	A	C	20.8%	246	401	89	81	52.4%	47.6%	Non-synonymous mutation	UL39
DISS29	81111	C	G	20.1%	256	398	86	78	52.4%	47.6%	Synonymous mutation	UL39
DISS29	81126	G	T	20.2%	287	390	103	68	60.2%	39.8%	Synonymous mutation	UL39
DISS29	81132	G	A	20.1%	299	390	106	67	61.3%	38.7%	Synonymous mutation	UL39
DISS29	81135	C	T	19.7%	306	385	106	63	62.7%	37.3%	Synonymous mutation	UL39
DISS29	81137	G	A	19.8%	314	389	109	65	62.6%	37.4%	Non-synonymous mutation	UL39
DISS29	81149	C	A	20.9%	331	379	129	58	69.0%	31.0%	Non-synonymous mutation	UL39
DISS29	81174	C	G	20.6%	362	385	143	51	73.7%	26.3%	Synonymous mutation	UL39
DISS29	81175	G	T	20.9%	366	381	145	52	73.6%	26.4%	Non-synonymous mutation	UL39
DISS29	81180	C	G	20.8%	384	373	149	50	74.9%	25.1%	Synonymous mutation	UL39
DISS29	81192	T	C	21.8%	412	351	160	53	75.1%	24.9%	Synonymous mutation	UL39
DISS29	81216	G	C	23.1%	437	339	185	48	79.4%	20.6%	Synonymous mutation	UL39
DISS29	81227	G	A	24.1%	452	331	199	50	79.9%	20.1%	Non-synonymous mutation	UL39
DISS29	81249	C	T	25.4%	492	338	223	59	79.1%	20.9%	Synonymous mutation	UL39
DISS29	81252	G	C	25.5%	495	344	225	62	78.4%	21.6%	Synonymous mutation	UL39
DISS29	81280	T	C	26.2%	520	367	243	73	76.9%	23.1%	Non-synonymous mutation	UL39
DISS29	81283	A	G	26.4%	513	376	243	76	76.2%	23.8%	Non-synonymous mutation	UL39
DISS29	81289	A	C	26.3%	511	385	237	83	74.1%	25.9%	Non-synonymous mutation	UL39
DISS29	81291	C	G	26.7%	517	377	239	87	73.3%	26.7%	Non-synonymous mutation	UL39
DISS29	81738	G	A	9.0%	590	427	70	31	69.3%	30.7%	Synonymous mutation	UL39
DISS29	82369	C	G	9.8%	699	430	93	30	75.6%	24.4%	Synonymous mutation	UL40
DISS29	82789	A	G	11.0%	523	331	80	26	75.5%	24.5%	Synonymous mutation	UL40
DISS29	83410	G	A	8.2%	572	275	64	12	84.2%	15.8%	Synonymous mutation	UL41
DISS29	83420	G	T	8.4%	600	280	69	12	85.2%	14.8%	Synonymous mutation	UL41
DISS29	85668	A	G	8.7%	559	537	65	40	61.9%	38.1%	Non-synonymous mutation	UL42
DISS29	86191	C	G	33.2%	449	202	272	51	84.2%	15.8%	Non-synonymous mutation	UL42
DISS29	86260	T	C	32.6%	449	233	258	73	77.9%	22.1%	Non-synonymous mutation	UL42
DISS29	86357	A	G	3.1%	655	439	22	13	62.9%	37.1%	Synonymous mutation	UL42
DISS29	86555	G	C	8.8%	518	472	64	32	66.7%	33.3%	Synonymous mutation	UL42
DISS29	86681	A	T	24.3%	510	322	134	134	50.0%	50.0%	Intergenic	intergenic
DISS29	86960	C	G	13.6%	155	539	39	70	35.8%	64.2%	Synonymous mutation	UL43
DISS29	87317	C	T	20.6%	486	334	148	66	69.2%	30.8%	Synonymous mutation	UL43
DISS29	87729	G	A	2.2%	610	434	18	5	78.3%	21.7%	Non-synonymous mutation	UL43
DISS29	87997	A	G	2.7%	532	472	21	7	75.0%	25.0%	Non-synonymous mutation	UL43
DISS29	88093	C	T	25.8%	304	286	147	59	71.4%	28.6%	Non-synonymous mutation	UL43
DISS29	88639	A	C	17.1%	133	359	37	65	36.3%	63.7%	Non-synonymous mutation	UL44
DISS29	88784	T	C	9.0%	499	340	52	31	62.7%	37.3%	Non-synonymous mutation	UL44
DISS29	88922	G	A	14.0%	396	181	65	29	69.1%	30.9%	Non-synonymous mutation	UL44
DISS29	88996	C	G	39.3%	381	52	195	92	67.9%	32.1%	Non-synonymous mutation	UL44
DISS29	88997	C	G	39.4%	387	54	196	91	68.3%	31.7%	Non-synonymous mutation	UL44
DISS29	88998	G	T	39.1%	387	56	192	93	67.4%	32.6%	Synonymous mutation	UL44
DISS29	88999	A	C	39.6%	371	49	192	93	67.4%	32.6%	Non-synonymous mutation	UL44
DISS29	89000	C	G	40.0%	378	48	192	93	67.4%	32.6%	Non-synonymous mutation	UL44

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	89001	C	G	39.8%	379	50	193	93	67.5%	32.5%	Synonymous mutation	UL44
DISS29	89069	T	C	28.2%	434	373	219	98	69.1%	30.9%	Non-synonymous mutation	UL44
DISS29	89247	G	A	28.2%	538	274	258	62	80.6%	19.4%	Synonymous mutation	UL44
DISS29	89915	G	C	6.2%	28	438	24	7	77.4%	22.6%	Intergenic	intergenic
DISS29	89924	C	A	12.1%	30	428	10	53	15.9%	84.1%	Intergenic	intergenic
DISS29	90636	T	C	36.5%	284	358	243	126	65.9%	34.1%	Intergenic	intergenic
DISS29	90647	A	T	2.4%	466	432	14	9	60.9%	39.1%	Intergenic	intergenic
DISS29	90654	G	C	2.9%	476	469	20	8	71.4%	28.6%	Intergenic	intergenic
DISS29	90685	C	T	33.5%	287	367	215	114	65.3%	34.7%	Intergenic	intergenic
DISS29	90710	C	A	30.9%	305	367	183	122	60.0%	40.0%	Intergenic	intergenic
DISS29	90804	A	G	24.4%	403	327	140	95	59.6%	40.4%	Intergenic	intergenic
DISS29	91100	C	G	8.8%	373	552	51	39	56.7%	43.3%	Synonymous mutation	UL46
DISS29	91112	G	A	30.5%	241	462	172	137	55.7%	44.3%	Synonymous mutation	UL46
DISS29	91113	T	A	31.0%	233	458	172	139	55.3%	44.7%	Non-synonymous mutation	UL46
DISS29	91114	C	G	30.8%	242	463	173	141	55.1%	44.9%	Non-synonymous mutation	UL46
DISS29	91115	T	A	30.9%	238	452	170	140	54.8%	45.2%	Non-synonymous mutation	UL46
DISS29	91116	T	C	30.5%	240	463	170	141	54.7%	45.3%	Non-synonymous mutation	UL46
DISS29	91125	A	G	31.4%	233	460	183	135	57.5%	42.5%	Non-synonymous mutation	UL46
DISS29	91189	T	C	37.6%	179	349	221	97	69.5%	30.5%	Non-synonymous mutation	UL46
DISS29	91246	T	C	39.8%	135	326	203	103	66.3%	33.7%	Non-synonymous mutation	UL46
DISS29	91267	T	C	29.8%	156	324	131	73	64.2%	35.8%	Non-synonymous mutation	UL46
DISS29	91376	T	C	12.3%	136	204	38	10	79.2%	20.8%	Synonymous mutation	UL46
DISS29	91403	A	G	11.8%	104	179	27	11	71.1%	28.9%	Synonymous mutation	UL46
DISS29	91730	C	T	11.4%	548	181	62	32	66.0%	34.0%	Synonymous mutation	UL46
DISS29	92693	C	A	7.6%	748	402	65	29	69.1%	30.9%	Non-synonymous mutation	UL46
DISS29	93066	C	T	8.6%	649	481	83	23	78.3%	21.7%	Intergenic	intergenic
DISS29	93550	G	A	9.9%	397	381	58	27	68.2%	31.8%	Synonymous mutation	UL47
DISS29	93632	G	T	11.2%	432	345	66	32	67.3%	32.7%	Synonymous mutation	UL47
DISS29	94031	G	A	2.7%	683	385	19	11	63.3%	36.7%	Synonymous mutation	UL47
DISS29	94274	C	T	19.0%	531	397	158	61	72.1%	27.9%	Synonymous mutation	UL47
DISS29	94652	A	G	20.9%	470	423	159	77	67.4%	32.6%	Synonymous mutation	UL47
DISS29	94669	G	A	20.3%	449	432	149	75	66.5%	33.5%	Non-synonymous mutation	UL47
DISS29	94731	T	C	8.3%	478	453	57	27	67.9%	32.1%	Non-synonymous mutation	UL47
DISS29	94993	A	G	33.2%	213	307	188	70	72.9%	27.1%	Non-synonymous mutation	UL47
DISS29	95085	A	C	10.4%	415	274	64	16	80.0%	20.0%	Non-synonymous mutation	UL47
DISS29	95190	G	A	38.9%	226	190	195	70	73.6%	26.4%	Non-synonymous mutation	UL47
DISS29	95522	G	A	38.4%	354	122	233	64	78.5%	21.5%	Intergenic	intergenic
DISS29	95538	C	T	2.6%	547	171	17	2	89.5%	10.5%	Intergenic	intergenic
DISS29	95621	A	C	15.5%	244	234	48	40	54.5%	45.5%	Intergenic	intergenic
DISS29	95683	T	C	30.6%	237	323	155	96	61.8%	38.2%	Intergenic	intergenic
DISS29	95743	T	G	27.1%	179	402	133	83	61.6%	38.4%	Intergenic	intergenic
DISS29	96521	G	A	30.0%	184	495	155	136	53.3%	46.7%	Synonymous mutation	UL48
DISS29	97522	C	T	26.9%	287	310	158	62	71.8%	28.2%	Non-synonymous mutation	UL48
DISS29	97570	C	T	12.7%	393	282	72	26	73.5%	26.5%	Intergenic	intergenic
DISS29	97822	T	C	5.4%	78	98	8	2	80.0%	20.0%	Intergenic	intergenic
DISS29	98518	G	A	43.8%	82	215	152	82	65.0%	35.0%	Non-synonymous mutation	UL49
DISS29	98711	A	G	39.4%	399	34	245	37	86.9%	13.1%	Synonymous mutation	UL49
DISS29	98762	C	T	35.7%	497	81	279	43	86.6%	13.4%	Synonymous mutation	UL49
DISS29	98969	G	C	36.5%	436	309	321	107	75.0%	25.0%	Intergenic	intergenic
DISS29	99198	A	G	24.3%	478	303	180	72	71.4%	28.6%	Intergenic	intergenic
DISS29	99430	C	T	9.3%	647	380	73	32	69.5%	30.5%	Synonymous mutation	UL49A
DISS29	99908	A	G	31.3%	453	348	241	125	65.8%	34.2%	Non-synonymous mutation	UL50
DISS29	100400	C	G	24.9%	613	347	235	83	73.9%	26.1%	Non-synonymous mutation	UL50
DISS29	100442	G	C	8.8%	717	424	77	34	69.4%	30.6%	Non-synonymous mutation	UL50
DISS29	100675	G	A	25.3%	405	375	196	68	74.2%	25.8%	Intergenic	intergenic
DISS29	100889	T	C	37.0%	407	202	264	96	73.3%	26.7%	Synonymous mutation	UL51
DISS29	101619	C	T	35.5%	331	426	286	131	68.6%	31.4%	Intergenic	intergenic
DISS29	101909	C	T	34.2%	400	317	283	90	75.9%	24.1%	Synonymous mutation	UL52
DISS29	102156	A	G	37.9%	489	240	313	131	70.5%	29.5%	Non-synonymous mutation	UL52
DISS29	102636	A	G	31.0%	524	348	297	95	75.8%	24.2%	Non-synonymous mutation	UL52
DISS29	102860	C	T	2.4%	762	506	23	8	74.2%	25.8%	Synonymous mutation	UL52
DISS29	103120	C	G	3.9%	161	552	14	15	48.3%	51.7%	Non-synonymous mutation	UL52
DISS29	103726	T	C	13.6%	736	245	124	30	80.5%	19.5%	Non-synonymous mutation	UL52
DISS29	104048	C	T	3.5%	797	545	29	19	60.4%	39.6%	Synonymous mutation	UL52
DISS29	104225	C	T	10.0%	576	523	75	47	61.5%	38.5%	Synonymous mutation	UL52
DISS29	104418	G	A	7.7%	822	452	75	32	70.1%	29.9%	Non-synonymous mutation	UL52
DISS29	104498	T	C	29.6%	570	376	255	143	64.1%	35.9%	Synonymous mutation	UL52
DISS29	104585	C	A	15.7%	633	449	92	109	45.8%	54.2%	Synonymous mutation	UL52
DISS29	104895	G	A	8.6%	715	398	79	26	75.2%	24.8%	Non-synonymous mutation	UL53
DISS29	105527	C	T	32.8%	502	196	281	60	82.4%	17.6%	Non-synonymous mutation	UL53
DISS29	105567	C	G	20.5%	597	157	175	20	89.7%	10.3%	Non-synonymous mutation	UL53
DISS29	105609	G	A	30.0%	548	344	264	119	68.9%	31.1%	Non-synonymous mutation	UL53
DISS29	105651	G	A	2.4%	814	432	20	11	64.5%	35.5%	Non-synonymous mutation	UL53
DISS29	105706	T	G	2.2%	843	456	22	7	75.9%	24.1%	Synonymous mutation	UL53
DISS29	105754	C	T	27.1%	587	405	283	85	76.9%	23.1%	Synonymous mutation	UL53
DISS29	105882	G	A	2.3%	816	486	21	9	70.0%	30.0%	Intergenic	intergenic
DISS29	105916	A	G	42.7%	59	462	288	100	74.2%	25.8%	Intergenic	intergenic
DISS29	105960	C	T	39.0%	167	457	312	88	78.0%	22.0%	Intergenic	intergenic
DISS29	106097	T	C	34.3%	449	339	305	107	74.0%	26.0%	Intergenic	intergenic
DISS29	106752	G	A	33.6%	297	368	235	103	69.5%	30.5%	Synonymous mutation	UL54
DISS29	107894	G	A	2.6%	607	382	18	8	69.2%	30.8%	Non-synonymous mutation	UL54
DISS29	107906	T	C	7.0%	590	337	42	28	60.0%	40.0%	Non-synonymous mutation	UL54
DISS29	108497	G	T	9.4%	68	203	16	12	57.1%	42.9%	Non-synonymous mutation	UL54
DISS29	109637	A	G	37.1%	421	275	312	99	75.9%	24.1%	Intergenic	intergenic
DISS29	109848	A	G	26.1%	199	376	127	76	62.6%	37.4%	Synonymous mutation	UL56
DISS29	110611	C	T	2.5%	80	229	1	7	12.5%	87.5%	Intergenic	intergenic
DISS29	110694	C	A	13.9%	40	170	21	13	61.8%	38.2%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	110932	T	C	15.2%	512	35	72	26	73.5%	26.5%	Intergenic	intergenic
DISS29	110945	G	A	7.4%	522	55	36	10	78.3%	21.7%	Intergenic	intergenic
DISS29	110960	G	C	3.4%	553	75	13	9	59.1%	40.9%	Intergenic	intergenic
DISS29	110961	G	A	7.1%	530	70	36	10	78.3%	21.7%	Intergenic	intergenic
DISS29	110964	C	G	4.6%	557	85	24	7	77.4%	22.6%	Intergenic	intergenic
DISS29	110966	G	C	6.0%	529	80	30	10	75.0%	25.0%	Intergenic	intergenic
DISS29	111008	C	G	13.5%	313	98	25	39	39.1%	60.9%	Intergenic	intergenic
DISS29	111010	C	G	5.5%	344	137	17	11	60.7%	39.3%	Intergenic	intergenic
DISS29	111687	T	C	29.5%	216	35	83	22	79.0%	21.0%	Intergenic	intergenic
DISS29	111700	G	A	15.5%	243	58	46	9	83.6%	16.4%	Intergenic	intergenic
DISS29	111710	G	C	2.5%	271	68	7	2	77.8%	22.2%	Intergenic	intergenic
DISS29	111715	G	C	4.6%	280	71	14	3	82.4%	17.6%	Intergenic	intergenic
DISS29	111716	G	A	9.3%	263	69	30	4	88.2%	11.8%	Intergenic	intergenic
DISS29	111718	C	A	2.3%	299	76	8	1	88.9%	11.1%	Intergenic	intergenic
DISS29	111719	C	G	4.6%	299	78	15	3	83.3%	16.7%	Intergenic	intergenic
DISS29	111721	G	C	5.3%	280	83	16	5	76.2%	23.8%	Intergenic	intergenic
DISS29	111722	G	C	8.9%	262	75	24	11	68.6%	31.4%	Intergenic	intergenic
DISS29	111733	A	G	14.1%	236	86	47	6	88.7%	11.3%	Intergenic	intergenic
DISS29	111736	G	C	3.7%	265	93	8	6	57.1%	42.9%	Intergenic	intergenic
DISS29	111737	G	C	10.9%	230	89	36	5	87.8%	12.2%	Intergenic	intergenic
DISS29	111739	G	A	7.3%	233	103	22	5	81.5%	18.5%	Intergenic	intergenic
DISS29	111740	G	C	8.5%	240	106	26	6	81.3%	18.8%	Intergenic	intergenic
DISS29	111741	G	A	6.9%	229	96	20	5	80.0%	20.0%	Intergenic	intergenic
DISS29	111742	A	C	8.0%	216	107	23	5	82.1%	17.9%	Intergenic	intergenic
DISS29	111743	T	C	10.5%	211	95	27	9	75.0%	25.0%	Intergenic	intergenic
DISS29	111745	G	A	5.6%	212	99	15	4	78.9%	21.1%	Intergenic	intergenic
DISS29	111752	G	C	2.5%	209	107	4	4	50.0%	50.0%	Intergenic	intergenic
DISS29	111753	A	C	2.2%	207	108	3	4	42.9%	57.1%	Intergenic	intergenic
DISS29	111755	G	C	3.7%	203	110	8	4	66.7%	33.3%	Intergenic	intergenic
DISS29	111757	A	C	4.1%	193	108	9	4	69.2%	30.8%	Intergenic	intergenic
DISS29	111758	G	C	4.0%	195	118	9	4	69.2%	30.8%	Intergenic	intergenic
DISS29	111761	G	C	2.1%	195	128	6	1	85.7%	14.3%	Intergenic	intergenic
DISS29	111763	C	G	28.6%	103	91	51	27	65.4%	34.6%	Intergenic	intergenic
DISS29	111765	C	G	16.4%	162	118	43	12	78.2%	21.8%	Intergenic	intergenic
DISS29	112136	G	C	43.1%	25	53	25	34	42.4%	57.6%	Intergenic	intergenic
DISS29	112172	A	G	50.9%	26	25	8	50	13.8%	86.2%	Intergenic	intergenic
DISS29	112237	G	A	19.2%	44	171	17	34	33.3%	66.7%	Intergenic	intergenic
DISS29	112368	T	C	2.4%	590	8	13	2	86.7%	13.3%	Intergenic	intergenic
DISS29	112488	C	T	15.2%	797	98	160	24	87.0%	13.0%	Intergenic	intergenic
DISS29	112489	C	G	16.1%	801	109	154	44	77.8%	22.2%	Intergenic	intergenic
DISS29	112490	C	G	25.8%	805	104	261	59	81.6%	18.4%	Intergenic	intergenic
DISS29	112492	C	G	26.8%	807	101	265	68	79.6%	20.4%	Intergenic	intergenic
DISS29	112494	T	G	27.0%	805	99	273	70	79.6%	20.4%	Intergenic	intergenic
DISS29	112496	C	G	28.9%	811	96	279	93	75.0%	25.0%	Intergenic	intergenic
DISS29	112497	C	G	29.0%	812	97	281	95	74.7%	25.3%	Intergenic	intergenic
DISS29	112499	C	G	11.8%	966	167	125	27	82.2%	17.8%	Intergenic	intergenic
DISS29	112719	G	T	12.1%	427	709	91	66	58.0%	42.0%	Intergenic	intergenic
DISS29	112803	T	G	8.8%	83	729	25	54	31.6%	68.4%	Intergenic	intergenic
DISS29	112984	T	G	21.5%	60	44	17	12	58.6%	41.4%	Intergenic	intergenic
DISS29	113191	C	G	9.0%	99	32	10	3	76.9%	23.1%	Intergenic	intergenic
DISS29	113195	G	C	6.7%	97	27	5	4	55.6%	44.4%	Intergenic	intergenic
DISS29	113196	T	G	8.3%	95	25	7	4	63.6%	36.4%	Intergenic	intergenic
DISS29	113239	G	C	7.5%	77	21	6	2	75.0%	25.0%	Intergenic	intergenic
DISS29	113348	G	C	34.8%	143	109	62	73	45.9%	54.1%	Intergenic	intergenic
DISS29	113437	G	C	2.7%	192	130	4	5	44.4%	55.6%	Intergenic	intergenic
DISS29	113439	C	G	3.9%	171	125	6	6	50.0%	50.0%	Intergenic	intergenic
DISS29	113471	C	G	13.9%	109	33	15	11	57.7%	42.3%	Intergenic	intergenic
DISS29	113472	C	T	28.6%	86	11	25	29	46.3%	53.7%	Intergenic	intergenic
DISS29	113473	G	A	16.8%	102	34	20	12	62.5%	37.5%	Intergenic	intergenic
DISS29	113474	C	T	21.1%	105	20	18	21	46.2%	53.8%	Intergenic	intergenic
DISS29	113475	G	T	20.6%	90	5	15	19	44.1%	55.9%	Intergenic	intergenic
DISS29	113476	C	T	4.9%	123	26	7	1	87.5%	12.5%	Intergenic	intergenic
DISS29	113477	G	T	15.4%	108	6	15	10	60.0%	40.0%	Intergenic	intergenic
DISS29	113478	T	C	14.9%	133	14	13	13	50.0%	50.0%	Intergenic	intergenic
DISS29	113479	T	C	13.6%	133	18	13	11	54.2%	45.8%	Intergenic	intergenic
DISS29	113480	C	T	9.9%	133	21	7	10	41.2%	58.8%	Intergenic	intergenic
DISS29	113483	G	C	7.9%	122	8	7	5	58.3%	41.7%	Intergenic	intergenic
DISS29	113513	T	G	3.2%	226	16	4	4	50.0%	50.0%	Intergenic	intergenic
DISS29	113516	T	G	8.1%	229	17	13	9	59.1%	40.9%	Intergenic	intergenic
DISS29	113518	C	G	11.1%	241	19	20	13	60.6%	39.4%	Intergenic	intergenic
DISS29	113519	T	G	8.4%	238	19	17	9	65.4%	34.6%	Intergenic	intergenic
DISS29	113521	A	G	12.7%	273	27	30	14	68.2%	31.8%	Intergenic	intergenic
DISS29	113524	C	G	23.4%	252	21	67	26	72.0%	28.0%	Intergenic	intergenic
DISS29	113530	G	A	30.5%	19	0	99	36	73.3%	26.7%	Intergenic	intergenic
DISS29	113671	T	C	34.3%	508	195	217	150	59.1%	40.9%	Intergenic	intergenic
DISS29	113702	A	G	32.7%	475	146	183	118	60.8%	39.2%	Intergenic	intergenic
DISS29	113705	A	G	31.8%	481	137	182	106	63.2%	36.8%	Intergenic	intergenic
DISS29	113742	T	C	9.9%	200	182	30	12	71.4%	28.6%	Intergenic	intergenic
DISS29	113791	C	T	5.0%	23	238	5	9	35.7%	64.3%	Intergenic	intergenic
DISS29	113887	G	A	33.2%	8	157	16	66	19.5%	80.5%	Intergenic	intergenic
DISS29	113904	T	C	20.5%	5	157	8	34	19.0%	81.0%	Intergenic	intergenic
DISS29	113932	G	T	41.1%	1	131	15	84	15.2%	84.8%	Intergenic	intergenic
DISS29	113934	G	C	48.0%	0	122	18	102	15.0%	85.0%	Intergenic	intergenic
DISS29	113969	C	T	2.0%	198	143	5	2	71.4%	28.6%	Intergenic	intergenic
DISS29	113972	A	C	5.8%	135	133	15	2	88.2%	11.8%	Intergenic	intergenic
DISS29	114059	G	A	58.8%	261	31	287	132	68.5%	31.5%	Intergenic	intergenic
DISS29	114076	T	C	44.0%	358	73	253	85	74.9%	25.1%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	114122	G	A	3.8%	692	220	20	16	55.6%	44.4%	Intergenic	intergenic
DISS29	114128	A	G	3.6%	625	231	26	6	81.3%	18.8%	Intergenic	intergenic
DISS29	114521	G	T	53.9%	329	290	485	241	66.8%	33.2%	Intergenic	intergenic
DISS29	114548	T	C	30.8%	564	501	307	167	64.8%	35.2%	Intergenic	intergenic
DISS29	114686	C	A	3.8%	83	393	13	6	68.4%	31.6%	Intergenic	intergenic
DISS29	114745	A	C	27.3%	159	476	63	179	26.0%	74.0%	Intergenic	intergenic
DISS29	114796	C	T	45.6%	176	326	229	194	54.1%	45.9%	Intergenic	intergenic
DISS29	115203	T	C	24.7%	231	508	84	160	34.4%	65.6%	Intergenic	intergenic
DISS29	115269	T	C	33.2%	456	595	276	248	52.7%	47.3%	Intergenic	intergenic
DISS29	115304	T	C	31.5%	597	627	347	219	61.3%	38.7%	Non-synonymous mutation	RL2
DISS29	115697	A	G	9.0%	949	782	133	39	77.3%	22.7%	Non-synonymous mutation	RL2
DISS29	115880	C	T	8.1%	295	777	10	84	10.6%	89.4%	Non-synonymous mutation	RL2
DISS29	115914	C	A	15.3%	128	658	59	84	41.3%	58.7%	Non-synonymous mutation	RL2
DISS29	115920	C	A	23.8%	80	559	84	124	40.4%	59.6%	Non-synonymous mutation	RL2
DISS29	115924	G	A	20.3%	81	587	73	98	42.7%	57.3%	Synonymous mutation	RL2
DISS29	115926	A	C	6.8%	130	621	15	40	27.3%	72.7%	Non-synonymous mutation	RL2
DISS29	115930	G	A	5.8%	126	604	14	31	31.1%	68.9%	Synonymous mutation	RL2
DISS29	115932	A	C	2.1%	131	607	5	11	31.3%	68.8%	Non-synonymous mutation	RL2
DISS29	115935	A	C	6.9%	107	562	19	31	38.0%	62.0%	Non-synonymous mutation	RL2
DISS29	115939	A	G	5.2%	111	550	10	26	27.8%	72.2%	Synonymous mutation	RL2
DISS29	115941	A	C	2.5%	114	542	4	13	23.5%	76.5%	Non-synonymous mutation	RL2
DISS29	115944	A	C	3.5%	97	507	8	14	36.4%	63.6%	Non-synonymous mutation	RL2
DISS29	115947	C	A	5.2%	87	472	15	16	48.4%	51.6%	Non-synonymous mutation	RL2
DISS29	115950	C	A	4.7%	82	461	13	14	48.1%	51.9%	Non-synonymous mutation	RL2
DISS29	115953	C	A	4.1%	81	434	8	14	36.4%	63.6%	Non-synonymous mutation	RL2
DISS29	115954	G	A	2.3%	86	431	3	9	25.0%	75.0%	Synonymous mutation	RL2
DISS29	115956	C	A	3.0%	78	415	5	10	33.3%	66.7%	Non-synonymous mutation	RL2
DISS29	116317	T	A	4.0%	170	49	3	6	33.3%	66.7%	Synonymous mutation	RL2
DISS29	116676	C	T	27.6%	129	13	44	11	80.0%	20.0%	Non-synonymous mutation	RL2
DISS29	116985	G	A	47.0%	133	7	104	20	83.9%	16.1%	Intergenic	RL2
DISS29	117205	T	G	36.8%	820	137	494	67	88.1%	11.9%	Non-synonymous mutation	RL2
DISS29	117242	C	A	35.3%	992	205	559	96	85.3%	14.7%	Non-synonymous mutation	RL2
DISS29	117245	G	A	5.0%	1466	294	79	13	85.9%	14.1%	Non-synonymous mutation	RL2
DISS29	117531	G	A	17.0%	1224	793	263	149	63.8%	36.2%	Synonymous mutation	RL2
DISS29	117779	T	C	20.6%	976	887	313	170	64.8%	35.2%	Intergenic	RL2
DISS29	118009	G	A	29.2%	718	650	347	217	61.5%	38.5%	Intergenic	intergenic
DISS29	118055	T	C	21.4%	874	621	242	166	59.3%	40.7%	Intergenic	intergenic
DISS29	118315	T	G	9.9%	1208	818	170	53	76.2%	23.8%	Intergenic	intergenic
DISS29	118318	T	C	9.8%	1245	807	171	51	77.0%	23.0%	Intergenic	intergenic
DISS29	118881	G	C	48.2%	279	138	299	91	76.7%	23.3%	Intergenic	intergenic
DISS29	119289	C	T	35.9%	132	37	58	39	59.8%	40.2%	Synonymous mutation	RL1
DISS29	119295	T	C	3.7%	188	71	8	2	80.0%	20.0%	Synonymous mutation	RL1
DISS29	119357	C	G	37.3%	154	29	93	16	85.3%	14.7%	Non-synonymous mutation	RL1
DISS29	119570	A	G	44.7%	46	5	23	19	54.8%	45.2%	Intergenic	RL1
DISS29	119588	T	C	31.8%	89	12	16	31	34.0%	66.0%	Intergenic	RL1
DISS29	120029	A	C	11.4%	73	25	10	3	76.9%	23.1%	Synonymous mutation	RL1
DISS29	120030	C	G	11.1%	78	25	10	3	76.9%	23.1%	Non-synonymous mutation	RL1
DISS29	120038	G	C	21.2%	75	17	22	3	88.0%	12.0%	Synonymous mutation	RL1
DISS29	120046	C	G	39.2%	70	3	36	11	76.6%	23.4%	Non-synonymous mutation	RL1
DISS29	120197	C	T	25.4%	224	37	79	10	88.8%	11.2%	Intergenic	intergenic
DISS29	120257	T	C	9.2%	213	81	22	9	71.0%	29.0%	Intergenic	intergenic
DISS29	120271	T	C	23.4%	159	72	60	11	84.5%	15.5%	Intergenic	intergenic
DISS29	120287	C	G	46.0%	98	52	93	35	72.7%	27.3%	Intergenic	intergenic
DISS29	120309	T	G	9.6%	118	65	11	9	55.0%	45.0%	Intergenic	intergenic
DISS29	120567	T	C	8.9%	119	16	12	2	85.7%	14.3%	Intergenic	intergenic
DISS29	120615	A	G	26.9%	174	41	66	13	83.5%	16.5%	Intergenic	intergenic
DISS29	120627	C	G	27.9%	162	58	70	15	82.4%	17.6%	Intergenic	intergenic
DISS29	120642	A	T	10.7%	191	68	21	10	67.7%	32.3%	Intergenic	intergenic
DISS29	120790	T	C	47.4%	127	13	64	65	49.6%	50.4%	Intergenic	intergenic
DISS29	120881	C	G	4.3%	23	132	5	2	71.4%	28.6%	Intergenic	intergenic
DISS29	120898	C	G	49.0%	89	32	24	92	20.7%	79.3%	Intergenic	intergenic
DISS29	120912	G	A	49.5%	129	16	48	96	33.3%	66.7%	Intergenic	intergenic
DISS29	120996	C	T	48.5%	301	81	195	165	54.2%	45.8%	Intergenic	intergenic
DISS29	121030	T	G	14.5%	132	269	22	47	31.9%	68.1%	Intergenic	intergenic
DISS29	121035	A	C	3.5%	65	370	14	2	87.5%	12.5%	Intergenic	intergenic
DISS29	121152	A	G	40.3%	55	63	33	48	40.7%	59.3%	Non-synonymous mutation	RS1
DISS29	121153	G	A	26.4%	73	65	16	36	30.8%	69.2%	Synonymous mutation	RS1
DISS29	121222	C	T	37.0%	39	33	25	19	56.8%	43.2%	Synonymous mutation	RS1
DISS29	121874	G	C	2.0%	146	193	6	1	85.7%	14.3%	Non-synonymous mutation	RS1
DISS29	121882	A	C	2.5%	156	192	8	1	88.9%	11.1%	Synonymous mutation	RS1
DISS29	121888	C	T	2.2%	162	188	7	1	87.5%	12.5%	Synonymous mutation	RS1
DISS29	121976	G	C	2.2%	179	129	6	1	85.7%	14.3%	Non-synonymous mutation	RS1
DISS29	122830	G	C	11.9%	170	0	17	6	73.9%	26.1%	Non-synonymous mutation	RS1
DISS29	122849	T	C	2.8%	278	1	6	2	75.0%	25.0%	Non-synonymous mutation	RS1
DISS29	122852	T	C	3.0%	293	2	6	3	66.7%	33.3%	Non-synonymous mutation	RS1
DISS29	123158	C	G	2.1%	213	258	7	3	70.0%	30.0%	Non-synonymous mutation	RS1
DISS29	123596	G	C	2.5%	295	12	7	1	87.5%	12.5%	Non-synonymous mutation	RS1
DISS29	123767	T	C	41.3%	381	86	291	39	88.2%	11.8%	Non-synonymous mutation	RS1
DISS29	123985	C	T	13.9%	970	198	136	53	72.0%	28.0%	Synonymous mutation	RS1
DISS29	124172	G	T	15.1%	413	612	129	53	70.9%	29.1%	Non-synonymous mutation	RS1
DISS29	124382	T	G	13.0%	61	408	32	38	45.7%	54.3%	Non-synonymous mutation	RS1
DISS29	124385	T	G	3.7%	83	414	9	10	47.4%	52.6%	Non-synonymous mutation	RS1
DISS29	124388	T	G	2.8%	84	405	6	8	42.9%	57.1%	Non-synonymous mutation	RS1
DISS29	124874	C	T	2.3%	461	430	12	9	57.1%	42.9%	Intergenic	intergenic
DISS29	124875	G	C	2.4%	459	431	12	10	54.5%	45.5%	Intergenic	intergenic
DISS29	125112	A	G	7.8%	293	120	16	19	45.7%	54.3%	Intergenic	intergenic
DISS29	125113	T	G	7.6%	272	117	15	19	44.1%	55.9%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	125128	G	T	3.8%	493	226	24	4	85.7%	14.3%	Intergenic	intergenic
DISS29	125338	T	G	11.1%	791	170	107	13	89.2%	10.8%	Intergenic	intergenic
DISS29	125407	A	T	2.4%	571	114	12	5	70.6%	29.4%	Intergenic	intergenic
DISS29	125412	C	G	2.5%	574	82	2	15	11.8%	88.2%	Intergenic	intergenic
DISS29	125423	G	T	6.3%	507	135	38	6	86.4%	13.6%	Intergenic	intergenic
DISS29	125433	T	G	11.0%	342	378	61	28	68.5%	31.5%	Intergenic	intergenic
DISS29	125509	A	G	37.2%	155	689	253	247	50.6%	49.4%	Intergenic	intergenic
DISS29	126060	G	A	28.6%	961	454	469	98	82.7%	17.3%	Intergenic	intergenic
DISS29	126200	G	A	10.9%	1255	677	167	70	70.5%	29.5%	Intergenic	intergenic
DISS29	126238	T	C	18.4%	988	790	267	133	66.8%	33.3%	Intergenic	intergenic
DISS29	126565	A	C	24.4%	48	284	16	92	14.8%	85.2%	Intergenic	intergenic
DISS29	126570	A	C	31.7%	44	231	22	106	17.2%	82.8%	Intergenic	intergenic
DISS29	126578	A	C	26.0%	53	189	13	72	15.3%	84.7%	Intergenic	intergenic
DISS29	126583	A	C	35.1%	40	134	20	77	20.6%	79.4%	Intergenic	intergenic
DISS29	126591	A	C	30.9%	58	93	12	56	17.6%	82.4%	Intergenic	intergenic
DISS29	126596	C	A	44.4%	37	58	29	50	36.7%	63.3%	Intergenic	intergenic
DISS29	126604	A	C	36.2%	61	11	7	35	16.7%	83.3%	Intergenic	intergenic
DISS29	126617	A	C	22.2%	76	5	3	21	12.5%	87.5%	Intergenic	intergenic
DISS29	126630	A	C	7.9%	91	2	1	7	12.5%	87.5%	Intergenic	intergenic
DISS29	126643	A	C	8.0%	104	0	3	6	33.3%	66.7%	Intergenic	intergenic
DISS29	126682	A	C	4.1%	184	1	4	4	50.0%	50.0%	Intergenic	intergenic
DISS29	126695	A	C	2.9%	228	1	3	4	42.9%	57.1%	Intergenic	intergenic
DISS29	126708	A	C	2.4%	277	2	4	3	57.1%	42.9%	Intergenic	intergenic
DISS29	126726	C	A	9.9%	311	33	31	7	81.6%	18.4%	Intergenic	intergenic
DISS29	126732	G	C	9.8%	334	69	34	10	77.3%	22.7%	Intergenic	intergenic
DISS29	126733	G	C	10.1%	320	60	36	9	80.0%	20.0%	Intergenic	intergenic
DISS29	126818	C	T	30.2%	357	77	162	26	86.2%	13.8%	Intergenic	intergenic
DISS29	126871	C	T	11.8%	388	109	58	9	86.6%	13.4%	Non-synonymous mutation	US1
DISS29	126893	A	G	14.4%	172	254	34	39	46.6%	53.4%	Synonymous mutation	US1
DISS29	126983	G	A	23.4%	356	282	122	74	62.2%	37.8%	Synonymous mutation	US1
DISS29	127146	G	A	34.5%	369	303	236	118	66.7%	33.3%	Non-synonymous mutation	US1
DISS29	127166	C	A	30.9%	395	351	240	94	71.9%	28.1%	Synonymous mutation	US1
DISS29	127173	C	T	18.7%	498	382	146	57	71.9%	28.1%	Non-synonymous mutation	US1
DISS29	127175	T	C	32.6%	360	344	251	91	73.4%	26.6%	Synonymous mutation	US1
DISS29	127229	C	T	33.9%	287	403	279	76	78.6%	21.4%	Synonymous mutation	US1
DISS29	127342	C	T	2.4%	699	519	24	6	80.0%	20.0%	Non-synonymous mutation	US1
DISS29	127415	A	G	31.4%	524	331	305	88	77.6%	22.4%	Synonymous mutation	US1
DISS29	127826	C	T	23.6%	139	275	68	60	53.1%	46.9%	Synonymous mutation	US1
DISS29	128119	T	G	27.1%	419	113	178	20	89.9%	10.1%	Intergenic	intergenic
DISS29	128188	G	C	2.1%	280	224	4	7	36.4%	63.6%	Intergenic	intergenic
DISS29	128244	T	G	2.2%	325	255	5	8	38.5%	61.5%	Intergenic	intergenic
DISS29	128666	G	T	29.5%	457	336	249	83	75.0%	25.0%	Non-synonymous mutation	US2
DISS29	128806	A	G	2.2%	693	389	17	7	70.8%	29.2%	Synonymous mutation	US2
DISS29	128986	C	T	19.4%	539	246	149	40	78.8%	21.2%	Synonymous mutation	US2
DISS29	129324	A	G	2.4%	545	135	5	12	29.4%	70.6%	Intergenic	intergenic
DISS29	130347	T	C	32.1%	434	338	260	105	71.2%	28.8%	Non-synonymous mutation	US3
DISS29	130733	G	A	35.3%	81	406	92	174	34.6%	65.4%	Synonymous mutation	US3
DISS29	130778	A	C	35.0%	191	371	150	153	49.5%	50.5%	Synonymous mutation	US3
DISS29	131071	G	A	32.1%	494	320	297	87	77.3%	22.7%	Non-synonymous mutation	US4
DISS29	131241	C	T	28.5%	517	386	236	123	65.7%	34.3%	Synonymous mutation	US4
DISS29	131372	C	T	28.0%	478	425	257	94	73.2%	26.8%	Synonymous mutation	US4
DISS29	131399	G	C	27.7%	491	441	261	97	72.9%	27.1%	Synonymous mutation	US4
DISS29	131578	C	T	32.5%	419	342	257	111	69.8%	30.2%	Non-synonymous mutation	US4
DISS29	131630	G	T	23.2%	354	414	147	87	62.8%	37.2%	Synonymous mutation	US4
DISS29	132012	G	A	35.9%	171	371	167	137	54.9%	45.1%	Non-synonymous mutation	US4
DISS29	132114	G	A	37.4%	164	344	207	97	68.1%	31.9%	Non-synonymous mutation	US4
DISS29	132150	G	A	37.9%	118	386	173	135	56.2%	43.8%	Non-synonymous mutation	US4
DISS29	132245	G	C	2.9%	135	335	7	7	50.0%	50.0%	Synonymous mutation	US4
DISS29	132651	A	G	53.1%	57	111	61	130	31.9%	68.1%	Non-synonymous mutation	US4
DISS29	133146	C	T	50.0%	91	105	72	125	36.5%	63.5%	Intergenic	intergenic
DISS29	133301	G	T	33.2%	295	232	195	67	74.4%	25.6%	Intergenic	intergenic
DISS29	133418	T	C	37.4%	407	144	259	70	78.7%	21.3%	Non-synonymous mutation	US5
DISS29	133633	C	T	23.3%	238	468	100	115	46.5%	53.5%	Synonymous mutation	US5
DISS29	133694	G	C	22.3%	310	370	109	86	55.9%	44.1%	Intergenic	intergenic
DISS29	133735	G	A	7.7%	409	302	33	26	55.9%	44.1%	Intergenic	intergenic
DISS29	133845	A	G	20.7%	10	204	15	41	26.8%	73.2%	Intergenic	intergenic
DISS29	133846	A	G	20.4%	11	226	13	48	21.3%	78.7%	Intergenic	intergenic
DISS29	133868	A	G	38.5%	71	248	62	138	31.0%	69.0%	Intergenic	intergenic
DISS29	133888	T	G	35.9%	124	265	93	125	42.7%	57.3%	Intergenic	intergenic
DISS29	134424	C	T	30.3%	487	410	274	116	70.3%	29.7%	Synonymous mutation	US6
DISS29	134598	C	T	7.8%	672	461	73	23	76.0%	24.0%	Synonymous mutation	US6
DISS29	134817	C	T	9.7%	596	558	77	47	62.1%	37.9%	Synonymous mutation	US6
DISS29	134830	C	T	23.7%	80	566	61	140	30.3%	69.7%	Synonymous mutation	US6
DISS29	135155	T	C	27.0%	405	393	213	85	71.5%	28.5%	Non-synonymous mutation	US6
DISS29	135286	T	A	9.8%	575	514	71	47	60.2%	39.8%	Intergenic	intergenic
DISS29	135311	T	C	32.5%	441	407	258	151	63.1%	36.9%	Intergenic	intergenic
DISS29	135754	A	G	30.9%	480	113	237	28	89.4%	10.6%	Non-synonymous mutation	US7
DISS29	135956	G	T	19.5%	518	413	178	47	79.1%	20.9%	Non-synonymous mutation	US7
DISS29	136123	T	C	38.1%	35	334	23	205	10.1%	89.9%	Non-synonymous mutation	US7
DISS29	136187	C	G	2.7%	170	255	2	10	16.7%	83.3%	Non-synonymous mutation	US7
DISS29	136191	A	G	7.8%	155	246	30	4	88.2%	11.8%	Synonymous mutation	US7
DISS29	136203	A	C	10.4%	258	312	35	31	53.0%	47.0%	Non-synonymous mutation	US7
DISS29	136493	T	C	35.3%	334	308	219	131	62.6%	37.4%	Non-synonymous mutation	US7
DISS29	137501	G	C	37.1%	19	401	87	162	34.9%	65.1%	Non-synonymous mutation	US8
DISS29	138076	A	G	9.1%	841	431	84	44	65.6%	34.4%	Synonymous mutation	US8
DISS29	138980	A	G	11.0%	503	543	79	51	60.8%	39.2%	Synonymous mutation	US9
DISS29	139211	A	G	31.0%	442	366	273	90	75.2%	24.8%	Synonymous mutation	US9

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	SNP Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	139354	G	A	21.0%	370	383	125	76	62.2%	37.8%	Intergenic	intergenic
DISS29	139356	A	G	23.4%	331	332	128	75	63.1%	36.9%	Intergenic	intergenic
DISS29	139392	A	C	12.4%	399	343	86	35	71.1%	28.9%	Intergenic	intergenic
DISS29	139398	G	C	4.8%	511	433	23	25	47.9%	52.1%	Intergenic	intergenic
DISS29	139408	C	G	19.6%	392	379	103	85	54.8%	45.2%	Intergenic	intergenic
DISS29	139487	G	T	2.7%	346	386	17	3	85.0%	15.0%	Intergenic	intergenic
DISS29	139501	A	G	5.4%	405	333	17	25	40.5%	59.5%	Intergenic	intergenic
DISS29	139502	A	C	3.8%	400	339	15	14	51.7%	48.3%	Intergenic	intergenic
DISS29	139503	C	A	5.2%	415	332	17	24	41.5%	58.5%	Intergenic	intergenic
DISS29	139510	G	C	2.2%	426	333	11	6	64.7%	35.3%	Intergenic	intergenic
DISS29	139511	G	C	2.8%	420	330	16	6	72.7%	27.3%	Intergenic	intergenic
DISS29	139512	C	G	2.3%	441	324	11	7	61.1%	38.9%	Intergenic	intergenic
DISS29	139513	T	C	2.7%	414	318	17	4	81.0%	19.0%	Intergenic	intergenic
DISS29	139564	C	G	5.5%	396	422	35	13	72.9%	27.1%	Intergenic	intergenic
DISS29	139565	C	G	7.1%	392	410	42	19	68.9%	31.1%	Intergenic	intergenic
DISS29	139566	C	G	7.9%	393	382	47	22	68.1%	31.9%	Intergenic	intergenic
DISS29	139567	G	A	10.3%	390	402	38	53	41.8%	58.2%	Intergenic	intergenic
DISS29	139570	A	G	14.7%	356	379	54	75	41.9%	58.1%	Intergenic	intergenic
DISS29	139652	A	G	16.7%	441	274	87	57	60.4%	39.6%	Intergenic	intergenic
DISS29	140176	C	G	22.7%	612	364	218	69	76.0%	24.0%	Synonymous mutation	US12
DISS29	140250	G	T	2.0%	715	443	18	6	75.0%	25.0%	Non-synonymous mutation	US12
DISS29	140291	C	T	22.3%	492	353	169	74	69.5%	30.5%	Non-synonymous mutation	US12
DISS29	140483	T	G	21.0%	484	363	158	67	70.2%	29.8%	Non-synonymous mutation	US12
DISS29	140849	A	G	31.5%	434	273	262	66	79.9%	20.1%	Intergenic	intergenic
DISS29	141140	A	G	34.8%	22	208	81	42	65.9%	34.1%	Synonymous mutation	US10
DISS29	141163	A	C	23.1%	15	74	14	38	26.9%	73.1%	Non-synonymous mutation	US10
DISS29	141164	C	G	19.4%	31	88	13	29	31.0%	69.0%	Synonymous mutation	US10

Table S3. Minor variants -- SNPs and INDELS -- detected in neonatal HSV-2 genomes (two Excel tabs)

Isolate	INDEL Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
SEM02	15258	G	GT	4.2%	961	1219	56	41	57.7%	42.3%	Intergenic	intergenic
SEM02	20681	G	GC	2.8%	1490	1210	42	38	52.5%	47.5%	Intergenic	intergenic
SEM02	25377	TC	T	14.3%	700	111	54	103	34.4%	65.6%	Intergenic	intergenic
SEM02	25639	GT	G	18.9%	955	407	271	103	72.5%	27.5%	Intergenic	intergenic
SEM02	26974	G	GA	34.1%	685	675	399	497	44.5%	55.5%	Intergenic	intergenic
SEM02	39360	G	GT	4.7%	676	656	41	25	62.1%	37.9%	Intergenic	intergenic
SEM02	43555	TG	T	4.3%	292	282	9	17	34.6%	65.4%	Intergenic	intergenic
SEM02	43883	G	GC	2.5%	935	295	18	14	56.3%	43.8%	Intergenic	intergenic
SEM02	53423	C	CCCTTTGACGT	2.0%	345	1784	13	31	29.5%	70.5%	Intergenic	intergenic
SEM02	53424	C	CCTT	19.6%	254	1425	80	348	18.7%	81.3%	Intergenic	intergenic
SEM02	53429	G	GC	3.2%	293	1662	10	55	15.4%	84.6%	Intergenic	intergenic
SEM02	53432	A	ACGC	21.8%	178	1355	76	356	17.6%	82.4%	Intergenic	intergenic
SEM02	53441	G	GC	2.6%	224	1450	5	40	11.1%	88.9%	Intergenic	intergenic
SEM02	68196	CG	C	4.0%	1495	571	71	18	79.8%	20.2%	Frameshift mutation	UL36
SEM02	71620	AC	A	16.4%	284	149	65	32	67.0%	33.0%	Intergenic	intergenic
SEM02	75494	AC	A	4.3%	1191	840	51	42	54.8%	45.2%	Intergenic	intergenic
SEM02	76985	TC	T	10.1%	735	324	63	63	50.0%	50.0%	Intergenic	intergenic
SEM02	85745	T	TC	2.4%	1371	1015	32	27	54.2%	45.8%	Intergenic	intergenic
SEM02	89756	C	CA	2.2%	792	710	11	23	32.4%	67.6%	Intergenic	intergenic
SEM02	89809	G	GT	11.3%	783	681	133	75	63.9%	36.1%	Intergenic	intergenic
SEM02	99415	GT	G	10.7%	911	721	111	116	48.9%	51.1%	Intergenic	intergenic
SEM02	106800	AC	A	10.6%	745	662	37	143	20.6%	79.4%	Intergenic	intergenic
SEM02	108821	GC	G	5.4%	1065	62	57	11	83.8%	16.2%	Intergenic	intergenic
SEM02	109158	G	GC	6.0%	218	564	17	36	32.1%	67.9%	Intergenic	intergenic
SEM02	109614	TG	T	23.4%	778	68	293	40	88.0%	12.0%	Intergenic	intergenic
SEM02	110345	T	TC	11.1%	169	5	24	3	88.9%	11.1%	Intergenic	intergenic
SEM02	110436	C	CG	3.9%	655	158	27	7	79.4%	20.6%	Intergenic	intergenic
SEM02	110552	A	AG	3.3%	1114	277	40	9	81.6%	18.4%	Intergenic	intergenic
SEM02	110773	AC	A	7.1%	1142	512	93	34	73.2%	26.8%	Intergenic	intergenic
SEM02	110816	TC	T	9.2%	501	662	46	86	34.8%	65.2%	Intergenic	intergenic
SEM02	110909	AC	A	6.9%	637	929	47	77	37.9%	62.1%	Intergenic	intergenic
SEM02	111845	TC	T	5.5%	35	826	7	46	13.2%	86.8%	Intergenic	intergenic
SEM02	112038	TC	T	2.2%	2423	736	27	46	37.0%	63.0%	Intergenic	intergenic
SEM02	122811	C	CAAA	2.7%	3534	100	54	48	52.9%	47.1%	Intergenic	intergenic
SEM02	122818	G	GCACT	4.8%	3237	87	83	91	47.7%	52.3%	Intergenic	intergenic
SEM02	122824	C	CCTG	7.0%	3199	171	177	76	70.0%	30.0%	Intergenic	intergenic
SEM02	122837	G	GTCATCCC	12.0%	3036	124	240	212	53.1%	46.9%	Intergenic	intergenic
SEM02	122991	C	CAAAG	9.3%	1607	3499	458	68	87.1%	12.9%	Intergenic	intergenic
SEM02	126613	CG	C	16.6%	313	84	56	30	65.1%	34.9%	Intergenic	intergenic
SEM02	137700	C	CT	4.1%	670	624	37	20	64.9%	35.1%	Intergenic	intergenic
SEM02	137709	T	TG	2.3%	734	669	20	14	58.8%	41.2%	Intergenic	intergenic
SEM02	137763	C	CG	6.2%	712	496	46	39	54.1%	45.9%	Intergenic	intergenic
SEM02	137874	GC	G	16.8%	630	346	154	108	58.8%	41.2%	Intergenic	intergenic
SEM02	139481	A	AGACATGGTGTCCC	5.8%	12	170	2	16	11.1%	88.9%	Frameshift mutation	US10
CNS03	15255	G	GT	9.8%	596	506	78	57	57.8%	42.2%	Intergenic	intergenic
CNS03	20682	G	GC	2.2%	1285	1128	29	26	52.7%	47.3%	Intergenic	intergenic
CNS03	25387	TC	T	15.1%	474	149	28	93	23.1%	76.9%	Intergenic	intergenic
CNS03	25648	G	GT	6.5%	1159	532	95	25	79.2%	20.8%	Intergenic	intergenic
CNS03	26981	G	GA	7.4%	885	960	73	91	44.5%	55.5%	Intergenic	intergenic
CNS03	32755	TC	T	11.9%	474	566	40	117	25.5%	74.5%	Intergenic	intergenic
CNS03	39352	G	GT	8.0%	630	522	64	39	62.1%	37.9%	Intergenic	intergenic
CNS03	41572	AC	A	6.2%	711	1007	50	69	42.0%	58.0%	Intergenic	intergenic
CNS03	43870	GC	G	4.1%	705	231	32	10	76.2%	23.8%	Intergenic	intergenic
CNS03	49257	AC	A	2.5%	24	625	2	15	11.8%	88.2%	Intergenic	intergenic
CNS03	68475	CG	C	4.6%	1326	489	67	25	72.8%	27.2%	Frameshift mutation	UL36
CNS03	71900	AC	A	2.2%	721	347	19	6	76.0%	24.0%	Intergenic	intergenic
CNS03	71909	C	CA	3.7%	768	349	28	16	63.6%	36.4%	Intergenic	intergenic
CNS03	77250	TC	T	9.4%	821	296	60	59	50.4%	49.6%	Intergenic	intergenic
CNS03	89310	GA	G	9.2%	360	792	35	82	29.9%	70.1%	Intergenic	intergenic
CNS03	90095	G	GT	11.6%	728	597	114	78	59.4%	40.6%	Intergenic	intergenic
CNS03	107074	AC	A	4.8%	792	834	28	57	32.9%	67.1%	Intergenic	intergenic
CNS03	109788	AC	A	10.6%	219	78	31	6	83.8%	16.2%	Intergenic	intergenic
CNS03	110137	AG	A	2.3%	271	406	7	9	43.8%	56.3%	Intergenic	intergenic
CNS03	110262	G	GC	21.5%	154	176	52	81	39.1%	60.9%	Intergenic	intergenic
CNS03	111528	CG	C	11.5%	263	32	37	6	86.0%	14.0%	Intergenic	intergenic
CNS03	111647	AG	A	12.4%	479	101	68	27	71.6%	28.4%	Intergenic	intergenic
CNS03	111913	TC	T	14.7%	187	286	29	57	33.7%	66.3%	Intergenic	intergenic
CNS03	111990	AC	A	31.0%	299	261	136	183	42.6%	57.4%	Intergenic	intergenic
CNS03	113035	G	GT	8.7%	1674	755	168	78	68.3%	31.7%	Intergenic	intergenic
CNS03	125681	AC	A	2.4%	942	946	25	23	52.1%	47.9%	Frameshift mutation	US1
CNS03	136007	T	TC	2.2%	191	796	17	5	77.3%	22.7%	Frameshift mutation	US8
CNS03	137910	C	CG	4.2%	913	745	46	29	61.3%	38.7%	Intergenic	intergenic
CNS03	138020	G	GC	10.2%	988	580	127	66	65.8%	34.2%	Intergenic	intergenic
CNS03	139294	T	TG	3.5%	975	535	36	21	63.2%	36.8%	Intergenic	intergenic
CNS03	139624	C	CCAAGA	2.7%	14	310	1	8	11.1%	88.9%	Frameshift mutation	US10
CNS11	8834	TG	T	3.4%	422	266	6	19	24.0%	76.0%	Intergenic	intergenic
CNS11	15264	G	GT	3.8%	913	1159	39	44	47.0%	53.0%	Intergenic	intergenic
CNS11	20686	G	GC	6.3%	1277	1113	77	94	45.0%	55.0%	Intergenic	intergenic
CNS11	25644	G	GT	16.6%	818	532	217	107	67.0%	33.0%	Intergenic	intergenic
CNS11	32748	T	TC	13.0%	714	665	84	140	38.0%	63.0%	Intergenic	intergenic
CNS11	39343	G	GT	29.2%	349	423	201	153	57.0%	43.0%	Intergenic	intergenic
CNS11	39376	TC	T	3.0%	282	516	3	22	12.0%	88.0%	Intergenic	intergenic
CNS11	43536	TG	T	38.6%	111	150	83	88	49.0%	51.0%	Intergenic	intergenic
CNS11	43854	GC	G	10.1%	429	118	44	23	66.0%	34.0%	Intergenic	intergenic
CNS11	49389	AC	A	2.7%	250	387	2	16	11.0%	89.0%	Intergenic	intergenic
CNS11	68031	CG	C	2.3%	1351	542	36	10	78.0%	22.0%	Frameshift mutation	UL36
CNS11	75334	AC	A	8.8%	514	539	46	64	42.0%	58.0%	Intergenic	intergenic
CNS11	85590	T	TC	7.8%	1016	695	77	78	50.0%	50.0%	Intergenic	intergenic
CNS11	89608	C	CA	4.5%	783	545	39	25	61.0%	39.0%	Intergenic	intergenic
CNS11	89661	G	GT	30.7%	488	351	312	159	66.0%	34.0%	Intergenic	intergenic
CNS11	94585	T	TG	18.6%	621	481	156	127	55.0%	45.0%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	INDEL Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
CNS11	99264	G	GT	7.9%	1098	1053	84	102	45.0%	55.0%	Intergenic	intergenic
CNS11	106648	AC	A	5.5%	779	703	30	60	33.0%	67.0%	Intergenic	intergenic
CNS11	108730	AC	A	6.0%	203	52	13	5	72.0%	28.0%	Intergenic	intergenic
CNS11	108871	G	GC	2.3%	723	172	12	9	57.0%	43.0%	Intergenic	intergenic
CNS11	108911	G	GC	19.9%	447	192	117	69	63.0%	37.0%	Intergenic	intergenic
CNS11	109206	G	GC	6.5%	528	527	38	41	48.0%	52.0%	Intergenic	intergenic
CNS11	109297	G	GA	2.9%	17	277	6	3	67.0%	33.0%	Intergenic	intergenic
CNS11	110566	C	CG	3.9%	541	166	22	7	76.0%	24.0%	Intergenic	intergenic
CNS11	110682	A	AG	3.4%	951	294	34	11	76.0%	24.0%	Intergenic	intergenic
CNS11	110947	T	TC	3.8%	646	596	14	36	28.0%	72.0%	Intergenic	intergenic
CNS11	111038	A	AC	16.6%	486	469	104	137	43.0%	57.0%	Intergenic	intergenic
CNS11	111563	CCCT	C	2.1%	1007	1288	23	25	48.0%	52.0%	Intergenic	intergenic
CNS11	112182	T	TC	2.8%	1897	586	10	64	14.0%	86.0%	Intergenic	intergenic
CNS11	124689	AC	A	2.9%	929	849	36	18	67.0%	33.0%	Frameshift mutation	US1
CNS11	125766	CG	C	7.7%	459	210	34	25	58.0%	42.0%	Intergenic	intergenic
CNS11	136851	C	CT	3.6%	655	662	21	29	42.0%	58.0%	Intergenic	intergenic
CNS11	136860	TG	T	4.1%	675	718	30	32	48.0%	52.0%	Intergenic	intergenic
CNS11	137025	G	GC	7.2%	742	549	72	37	66.0%	34.0%	Intergenic	intergenic
CNS11	138299	T	TG	11.0%	620	158	107	17	86.0%	14.0%	Intergenic	intergenic
CNS11	138633	A	TCCCGTCCACGAAG	4.1%	15	205	2	11	15.0%	85.0%	Frameshift mutation	US10
CNS12	15255	G	GT	4.2%	596	797	34	28	54.8%	45.2%	Intergenic	intergenic
CNS12	20680	GC	G	2.1%	1165	956	25	22	53.2%	46.8%	Intergenic	intergenic
CNS12	25380	TC	T	2.6%	382	313	7	12	36.8%	63.2%	Intergenic	intergenic
CNS12	25638	G	GT	7.7%	916	389	89	27	76.7%	23.3%	Intergenic	intergenic
CNS12	26972	G	GA	5.6%	1111	885	71	50	58.7%	41.3%	Intergenic	intergenic
CNS12	32743	TC	T	4.6%	548	688	25	37	40.3%	59.7%	Intergenic	intergenic
CNS12	37143	TG	T	17.4%	867	263	177	99	64.1%	35.9%	Intergenic	intergenic
CNS12	39345	G	GT	3.9%	579	629	22	28	44.0%	56.0%	Intergenic	intergenic
CNS12	41560	AC	A	3.6%	653	1100	28	39	41.8%	58.2%	Intergenic	intergenic
CNS12	43863	G	GC	19.1%	242	35	24	51	32.0%	68.0%	Intergenic	intergenic
CNS12	71463	A	AC	3.0%	633	200	23	3	88.5%	11.5%	Intergenic	intergenic
CNS12	75332	AC	A	3.8%	448	667	21	24	46.7%	53.3%	Intergenic	intergenic
CNS12	76826	TC	T	4.4%	490	338	19	20	48.7%	51.3%	Intergenic	intergenic
CNS12	89594	TC	T	8.4%	235	365	25	33	43.1%	56.9%	Intergenic	intergenic
CNS12	89659	G	GT	5.9%	531	709	35	44	44.3%	55.7%	Intergenic	intergenic
CNS12	94583	T	TG	6.2%	591	384	38	34	52.8%	47.2%	Intergenic	intergenic
CNS12	108733	C	CCACCGGCGGGAT	3.0%	344	70	2	11	15.4%	84.6%	Intergenic	intergenic
CNS12	108752	AC	A	8.9%	166	73	15	12	55.6%	44.4%	Intergenic	intergenic
CNS12	108881	A	AGCGAG	5.4%	369	121	11	19	36.7%	63.3%	Intergenic	intergenic
CNS12	108893	G	GC	14.0%	393	204	64	38	62.7%	37.3%	Intergenic	intergenic
CNS12	109102	AG	A	4.4%	232	329	12	15	44.4%	55.6%	Intergenic	intergenic
CNS12	109228	GC	G	6.4%	136	412	8	33	19.5%	80.5%	Intergenic	intergenic
CNS12	109992	GC	G	9.9%	77	7	7	3	70.0%	30.0%	Intergenic	intergenic
CNS12	111254	CG	C	11.7%	226	21	35	4	89.7%	10.3%	Intergenic	intergenic
CNS12	111374	A	AG	4.4%	744	189	34	10	77.3%	22.7%	Intergenic	intergenic
CNS12	111638	TC	T	5.9%	205	562	13	38	25.5%	74.5%	Intergenic	intergenic
CNS12	111714	AC	A	4.2%	530	660	22	32	40.7%	59.3%	Intergenic	intergenic
CNS12	122335	ATCG	A	2.5%	1373	1023	34	28	54.8%	45.2%	Inframe deletion	RS1
CNS12	122864	CG	C	16.8%	593	260	114	68	62.6%	37.4%	Intergenic	intergenic
CNS12	123435	G	GTCATCCC	10.4%	2916	159	310	69	81.8%	18.2%	Intergenic	intergenic
CNS12	123589	C	CAAAG	7.9%	2365	3157	405	71	85.1%	14.9%	Intergenic	intergenic
CNS12	125526	C	CA	2.1%	647	236	10	9	52.6%	47.4%	Intergenic	intergenic
CNS12	134006	T	TC	3.4%	207	422	5	18	21.7%	78.3%	Intergenic	intergenic
CNS12	136686	TG	T	8.3%	347	365	35	34	50.7%	49.3%	Intergenic	intergenic
CNS12	136850	GC	G	22.1%	468	123	160	69	69.9%	30.1%	Intergenic	intergenic
SEM13	15255	G	GT	7.6%	404	557	45	38	54.2%	45.8%	Intergenic	intergenic
SEM13	20679	GC	G	12.1%	566	525	78	93	45.6%	54.4%	Intergenic	intergenic
SEM13	25634	G	GT	6.6%	860	469	70	28	71.4%	28.6%	Intergenic	intergenic
SEM13	26967	GA	G	8.9%	603	668	69	70	49.6%	50.4%	Intergenic	intergenic
SEM13	32739	TC	T	5.6%	429	533	39	21	65.0%	35.0%	Intergenic	intergenic
SEM13	43867	G	GC	2.5%	720	199	19	5	79.2%	20.8%	Intergenic	intergenic
SEM13	68028	C	CG	2.1%	801	383	17	9	65.4%	34.6%	Frameshift mutation	UL36
SEM13	75328	AC	A	3.8%	491	521	18	23	43.9%	56.1%	Intergenic	intergenic
SEM13	89665	G	GT	5.2%	827	771	52	37	58.4%	41.6%	Intergenic	intergenic
SEM13	94590	T	TG	2.0%	740	636	16	13	55.2%	44.8%	Intergenic	intergenic
SEM13	108644	C	CG	5.5%	698	66	38	10	79.2%	20.8%	Intergenic	intergenic
SEM13	108853	AG	A	4.6%	181	639	12	29	29.3%	70.7%	Intergenic	intergenic
SEM13	108979	G	GC	6.3%	105	403	10	27	27.0%	73.0%	Intergenic	intergenic
SEM13	109428	TG	T	11.9%	669	73	91	18	83.5%	16.5%	Intergenic	intergenic
SEM13	110365	A	AG	2.0%	689	207	16	3	84.2%	15.8%	Intergenic	intergenic
SEM13	110629	TC	T	14.1%	160	230	28	46	37.8%	62.2%	Intergenic	intergenic
SEM13	110723	AC	A	3.4%	571	576	19	23	45.2%	54.8%	Intergenic	intergenic
SEM13	111662	TC	T	2.3%	107	621	5	12	29.4%	70.6%	Intergenic	intergenic
SEM13	111771	G	GT	21.2%	1262	381	366	105	77.7%	22.3%	Intergenic	intergenic
SEM13	111855	T	TC	12.7%	932	114	25	139	15.2%	84.8%	Intergenic	intergenic
SEM13	121547	ATCG	A	2.3%	1092	587	21	18	53.8%	46.2%	Inframe deletion	RS1
SEM13	122112	TG	T	6.0%	343	121	27	6	81.8%	18.2%	Intergenic	intergenic
SEM13	122285	C	CG	16.1%	87	1013	21	192	9.9%	90.1%	Intergenic	intergenic
SEM13	124952	CG	C	12.1%	341	255	48	37	56.5%	43.5%	Intergenic	intergenic
SEM13	133476	CG	C	9.6%	557	179	63	24	72.4%	27.6%	Intergenic	intergenic
SEM13	136112	CG	C	10.4%	552	465	66	66	50.0%	50.0%	Intergenic	intergenic
SEM13	136228	CGG	C	9.3%	390	172	46	22	67.6%	32.4%	Intergenic	intergenic
DISS14	20676	G	GC	3.0%	1318	1069	39	36	52.0%	48.0%	Intergenic	intergenic
DISS14	26960	G	GA	3.8%	1197	1194	56	40	58.3%	41.7%	Intergenic	intergenic
DISS14	32731	T	TC	3.0%	910	895	22	35	38.6%	61.4%	Intergenic	intergenic
DISS14	39326	G	GT	3.9%	597	564	31	17	64.6%	35.4%	Intergenic	intergenic
DISS14	43520	TG	T	3.4%	184	231	7	8	46.7%	53.3%	Intergenic	intergenic
DISS14	43838	GC	G	5.4%	726	243	45	13	77.6%	22.4%	Intergenic	intergenic
DISS14	67998	CG	C	2.3%	1427	629	34	15	69.4%	30.6%	Frameshift mutation	UL36
DISS14	75090	G	GT	5.0%	222	318	17	12	58.6%	41.4%	Intergenic	intergenic
DISS14	75308	AC	A	19.3%	190	254	34	99	25.6%	74.4%	Intergenic	intergenic
DISS14	89583	C	CA	27.1%	637	769	248	336	42.5%	57.5%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	INDEL Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS14	89637	G	GT	2.3%	968	1021	29	18	61.7%	38.3%	Intergenic	intergenic
DISS14	94562	T	TG	3.7%	828	691	37	24	60.7%	39.3%	Intergenic	intergenic
DISS14	99238	G	GT	9.1%	821	749	105	73	59.0%	41.0%	Intergenic	intergenic
DISS14	99548	CG	C	2.3%	1161	892	26	24	52.0%	48.0%	Frameshift mutation	UL51
DISS14	108633	C	CTT	3.8%	154	20	6	1	85.7%	14.3%	Intergenic	intergenic
DISS14	108640	CCA	C	7.8%	198	26	15	4	78.9%	21.1%	Intergenic	intergenic
DISS14	108644	C	CGGCGGGA	11.2%	189	24	15	15	50.0%	50.0%	Intergenic	intergenic
DISS14	108653	A	AG	8.4%	243	33	8	19	29.6%	70.4%	Intergenic	intergenic
DISS14	108660	C	CA	3.5%	280	54	8	4	66.7%	33.3%	Intergenic	intergenic
DISS14	108661	C	CGGG	4.0%	280	48	4	10	28.6%	71.4%	Intergenic	intergenic
DISS14	108929	A	AGCGAG	6.6%	576	202	39	20	66.1%	33.9%	Intergenic	intergenic
DISS14	108941	G	GC	14.1%	633	307	128	33	79.5%	20.5%	Intergenic	intergenic
DISS14	109152	AG	A	2.0%	224	683	6	13	31.6%	68.4%	Intergenic	intergenic
DISS14	109277	G	GC	5.2%	62	670	5	37	11.9%	88.1%	Intergenic	intergenic
DISS14	111019	AC	A	12.9%	490	280	64	64	50.0%	50.0%	Intergenic	intergenic
DISS14	111910	TG	T	15.8%	156	552	19	114	14.3%	85.7%	Intergenic	intergenic
DISS14	112189	TC	T	18.2%	188	475	43	135	24.2%	75.8%	Intergenic	intergenic
DISS14	114746	C	CG	2.2%	114	1583	5	35	12.5%	87.5%	Intergenic	intergenic
DISS14	114962	G	GC	7.9%	1321	1202	126	102	55.3%	44.7%	Intergenic	intergenic
DISS14	117224	GT	G	9.8%	40	52	5	5	50.0%	50.0%	Intergenic	intergenic
DISS14	123135	TC	T	3.0%	274	1711	9	55	14.1%	85.9%	Intergenic	intergenic
DISS14	123376	A	AC	4.8%	103	56	1	7	12.5%	87.5%	Intergenic	intergenic
DISS14	123877	AC	A	2.5%	852	777	22	20	52.4%	47.6%	Frameshift mutation	US1
DISS14	124954	CG	C	3.3%	510	321	16	14	53.3%	46.7%	Intergenic	intergenic
DISS14	136048	TG	T	10.3%	629	552	82	65	55.8%	44.2%	Intergenic	intergenic
CNS15	15254	G	GT	3.3%	1674	1855	63	60	51.0%	49.0%	Intergenic	intergenic
CNS15	25420	G	GT	2.1%	1171	947	27	18	60.0%	40.0%	Intergenic	intergenic
CNS15	26968	G	GA	4.8%	2226	2127	118	115	51.0%	49.0%	Intergenic	intergenic
CNS15	32740	TC	T	13.1%	669	866	102	151	40.0%	60.0%	Intergenic	intergenic
CNS15	37143	TG	T	16.4%	1794	398	318	194	62.0%	38.0%	Intergenic	intergenic
CNS15	39346	G	GT	4.6%	1086	1185	55	56	50.0%	50.0%	Intergenic	intergenic
CNS15	39533	T	TC	2.3%	1768	968	44	21	68.0%	32.0%	Intergenic	intergenic
CNS15	41509	GC	G	2.4%	2406	1922	71	37	66.0%	34.0%	Intergenic	intergenic
CNS15	43693	AG	A	2.4%	310	38	7	2	78.0%	22.0%	Intergenic	intergenic
CNS15	49517	C	CCCGCCG	2.5%	1566	540	44	11	80.0%	20.0%	onservative inframe inserti	UL29
CNS15	59237	G	GCCCGCC	2.2%	2682	1506	58	38	60.0%	40.0%	Disruptive inframe insertior	UL32
CNS15	61923	TG	T	3.4%	264	1154	11	39	22.0%	78.0%	Intergenic	intergenic
CNS15	62499	G	GAGTTA	2.8%	82	391	4	10	29.0%	71.0%	Frameshift mutation	UL36
CNS15	62503	G	GC	2.1%	83	370	4	6	40.0%	60.0%	Frameshift mutation	UL36
CNS15	65550	GGGGCGC	G	2.4%	2468	2005	49	59	45.0%	55.0%	onservative inframe deleti	UL36
CNS15	68032	CG	C	4.6%	2761	1191	142	55	72.0%	28.0%	Frameshift mutation	UL36
CNS15	71456	AC	A	11.9%	770	347	111	50	69.0%	31.0%	Intergenic	intergenic
CNS15	75118	G	GT	2.8%	784	1253	12	46	21.0%	79.0%	Intergenic	intergenic
CNS15	75334	AC	A	8.1%	1082	1079	89	112	44.0%	56.0%	Intergenic	intergenic
CNS15	76999	AC	A	2.4%	2240	1422	56	34	62.0%	38.0%	Intergenic	intergenic
CNS15	85600	TC	T	15.0%	1087	457	105	215	33.0%	67.0%	Intergenic	intergenic
CNS15	89667	G	GT	11.4%	1292	690	222	81	73.0%	27.0%	Intergenic	intergenic
CNS15	91957	GC	G	2.3%	2629	2299	69	45	61.0%	39.0%	Intergenic	intergenic
CNS15	94597	T	TG	6.3%	1497	1103	106	85	55.0%	45.0%	Intergenic	intergenic
CNS15	99206	GT	G	8.0%	1806	1576	190	147	56.0%	44.0%	Intergenic	intergenic
CNS15	108615	C	CCACCGGCGGGAT	3.5%	1295	193	29	25	54.0%	46.0%	Intergenic	intergenic
CNS15	108633	C	CGAG	3.1%	888	302	28	11	72.0%	28.0%	Intergenic	intergenic
CNS15	108762	A	AGCGAG	9.9%	1211	455	148	64	70.0%	30.0%	Intergenic	intergenic
CNS15	108774	G	GC	23.2%	1265	648	462	140	77.0%	23.0%	Intergenic	intergenic
CNS15	108983	AG	A	4.2%	676	1558	34	64	35.0%	65.0%	Intergenic	intergenic
CNS15	109108	GC	G	5.0%	462	1395	26	79	25.0%	75.0%	Intergenic	intergenic
CNS15	109593	T	TG	3.8%	2895	329	121	13	90.0%	10.0%	Intergenic	intergenic
CNS15	109857	C	CT	2.6%	204	2841	9	74	11.0%	89.0%	Intergenic	intergenic
CNS15	110417	CG	C	9.5%	971	237	112	28	80.0%	20.0%	Intergenic	intergenic
CNS15	110535	A	AG	5.5%	1839	568	110	34	76.0%	24.0%	Intergenic	intergenic
CNS15	110616	CG	C	2.2%	3349	373	76	9	89.0%	11.0%	Intergenic	intergenic
CNS15	110798	TC	T	4.2%	1582	1598	68	79	46.0%	54.0%	Intergenic	intergenic
CNS15	110890	AC	A	3.0%	1319	2185	43	69	38.0%	62.0%	Intergenic	intergenic
CNS15	111015	C	CG	2.5%	344	2956	17	69	20.0%	80.0%	Intergenic	intergenic
CNS15	111409	C	CCCT	2.3%	1524	2149	36	53	40.0%	60.0%	Intergenic	intergenic
CNS15	111965	C	CCT	6.0%	133	132	8	9	47.0%	53.0%	Intergenic	intergenic
CNS15	112088	C	CGCCA	2.1%	1351	437	26	13	67.0%	33.0%	Intergenic	intergenic
CNS15	112332	GT	G	2.2%	3891	1710	92	36	72.0%	28.0%	Intergenic	intergenic
CNS15	112457	TC	T	3.7%	868	2228	48	72	40.0%	60.0%	Intergenic	intergenic
CNS15	113278	C	CG	2.2%	9	298	1	6	14.0%	86.0%	Frameshift mutation	RL2
CNS15	116589	G	GCCT	6.6%	165	113	18	2	90.0%	10.0%	onservative inframe inserti	RL1
CNS15	122413	G	GTCGGCA	2.6%	3248	2405	69	84	45.0%	55.0%	Disruptive inframe insertior	RS1
CNS15	122728	TG	T	12.6%	1460	324	205	93	69.0%	31.0%	Intergenic	intergenic
CNS15	125522	GA	G	2.9%	1435	545	47	12	80.0%	20.0%	Intergenic	intergenic
CNS15	125660	C	CG	4.2%	916	655	41	30	58.0%	42.0%	Intergenic	intergenic
CNS15	125930	CGCCGGG	C	2.0%	817	345	20	4	83.0%	17.0%	onservative inframe deleti	US2
CNS15	128308	AG	A	2.9%	2011	2103	56	67	46.0%	54.0%	Intergenic	intergenic
CNS15	130496	GGT	G	3.0%	836	919	31	23	57.0%	43.0%	Intergenic	intergenic
CNS15	131253	TG	T	15.1%	144	536	22	125	15.0%	85.0%	Intergenic	intergenic
CNS15	131490	GT	G	2.0%	2398	815	45	21	68.0%	32.0%	Intergenic	intergenic
CNS15	134182	CG	C	5.1%	1306	607	70	43	62.0%	38.0%	Intergenic	intergenic
CNS15	136763	TG	T	7.7%	939	1026	77	97	44.0%	56.0%	Intergenic	intergenic
CNS15	136778	GA	G	3.3%	916	1225	28	46	38.0%	62.0%	Intergenic	intergenic
CNS15	136815	A	AC	3.5%	980	957	34	37	48.0%	52.0%	Intergenic	intergenic
CNS15	136820	C	CG	2.6%	1016	988	32	22	59.0%	41.0%	Intergenic	intergenic
CNS15	136932	G	GC	19.2%	939	230	394	51	89.0%	11.0%	Intergenic	intergenic
CNS15	138540	G	TGGTGTCCCGTCC	3.3%	22	243	3	16	16.0%	84.0%	Frameshift mutation	US10
CNS17	15260	G	GT	4.5%	528	748	27	34	44.3%	55.7%	Intergenic	intergenic
CNS17	20684	G	GC	2.7%	1080	1066	31	30	50.8%	49.2%	Intergenic	intergenic
CNS17	25640	G	GT	8.5%	959	434	94	38	71.2%	28.8%	Intergenic	intergenic
CNS17	26973	G	GA	6.9%	906	945	67	77	46.5%	53.5%	Intergenic	intergenic
CNS17	32745	TC	T	9.7%	391	508	28	76	26.9%	73.1%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	INDEL Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
CNS17	37147	TG	T	3.9%	1177	929	53	35	60.2%	39.8%	Intergenic	intergenic
CNS17	39346	G	GT	11.0%	325	411	56	51	52.3%	47.7%	Intergenic	intergenic
CNS17	39380	TC	T	2.2%	120	319	4	6	40.0%	60.0%	Intergenic	intergenic
CNS17	41564	A	AC	2.2%	643	1075	8	31	20.5%	79.5%	Intergenic	intergenic
CNS17	43542	TG	T	9.4%	97	153	16	11	59.3%	40.7%	Intergenic	intergenic
CNS17	60508	AG	A	14.6%	703	79	138	22	86.3%	13.8%	Intergenic	intergenic
CNS17	71428	C	CT	2.1%	285	322	7	6	53.8%	46.2%	Intergenic	intergenic
CNS17	71461	AC	A	9.9%	250	97	33	8	80.5%	19.5%	Intergenic	intergenic
CNS17	75339	AC	A	3.8%	431	609	16	26	38.1%	61.9%	Intergenic	intergenic
CNS17	76834	TC	T	4.5%	473	330	20	19	51.3%	48.7%	Intergenic	intergenic
CNS17	85606	TC	T	17.9%	421	307	65	112	36.7%	63.3%	Intergenic	intergenic
CNS17	89673	G	GT	4.7%	765	826	43	37	53.8%	46.3%	Intergenic	intergenic
CNS17	94600	T	TG	7.0%	588	377	50	30	62.5%	37.5%	Intergenic	intergenic
CNS17	96176	AG	A	9.7%	57	47	9	4	69.2%	30.8%	Intergenic	intergenic
CNS17	99229	G	GT	2.7%	848	875	29	20	59.2%	40.8%	Intergenic	intergenic
CNS17	106611	AC	A	7.3%	433	587	25	60	29.4%	70.6%	Intergenic	intergenic
CNS17	108601	GC	G	6.2%	1003	114	51	28	64.6%	35.4%	Intergenic	intergenic
CNS17	108602	C	CCG	4.1%	965	106	37	13	74.0%	26.0%	Intergenic	intergenic
CNS17	108628	CG	C	2.2%	761	269	20	3	87.0%	13.0%	Intergenic	intergenic
CNS17	108813	AG	A	4.0%	183	775	8	33	19.5%	80.5%	Intergenic	intergenic
CNS17	108939	GC	G	13.3%	131	325	21	57	26.9%	73.1%	Intergenic	intergenic
CNS17	110326	AG	A	10.0%	464	135	60	12	83.3%	16.7%	Intergenic	intergenic
CNS17	110682	A	AC	3.5%	410	512	23	12	65.7%	34.3%	Intergenic	intergenic
CNS17	112778	G	GT	3.0%	1510	707	54	16	77.1%	22.9%	Intergenic	intergenic
CNS17	122638	TG	T	7.2%	693	226	52	28	65.0%	35.0%	Intergenic	intergenic
CNS17	132073	T	TG	4.4%	106	726	5	35	12.5%	87.5%	Intergenic	intergenic
CNS17	135735	AC	A	3.1%	36	583	2	19	9.5%	90.5%	Frameshift mutation	US8
CNS17	137568	C	CT	5.9%	443	621	35	33	51.5%	48.5%	Intergenic	intergenic
CNS17	137577	T	TG	2.7%	479	681	14	19	42.4%	57.6%	Intergenic	intergenic
CNS17	137632	C	CG	4.0%	549	577	26	22	54.2%	45.8%	Intergenic	intergenic
CNS17	137742	GC	G	13.6%	526	388	91	76	54.5%	45.5%	Intergenic	intergenic
CNS17	139349	G	GAC	4.5%	3	133	2	12	14.3%	85.7%	Frameshift mutation	US10
SEM18	25379	T	TC	19.9%	2157	498	293	408	41.8%	58.2%	Intergenic	intergenic
SEM18	25426	TC	T	16.4%	823	1434	209	301	41.0%	59.0%	Intergenic	intergenic
SEM18	25643	G	GT	7.7%	3886	1930	370	138	72.8%	27.2%	Intergenic	intergenic
SEM18	26976	AAAAAAAA	G	7.3%	1190	570	110	54	67.1%	32.9%	Intergenic	intergenic
SEM18	31306	C	CCAA	60.8%	91	578	118	925	11.3%	88.7%	Intergenic	intergenic
SEM18	31311	A	AACAGGCGAG	11.2%	156	1263	28	152	15.6%	84.4%	Intergenic	intergenic
SEM18	31316	A	AGGGGAGG	17.6%	70	669	24	195	11.0%	89.0%	Intergenic	intergenic
SEM18	32905	T	TC	10.3%	2079	2475	211	354	37.3%	62.7%	Intergenic	intergenic
SEM18	44031	GC	G	3.9%	2530	618	112	21	84.2%	15.8%	Intergenic	intergenic
SEM18	53573	CCTT	C	2.1%	14118	8089	82	403	16.9%	83.1%	Intergenic	intergenic
SEM18	62861	C	CAGGG	3.2%	449	5	13	2	86.7%	13.3%	Frameshift mutation	UL36
SEM18	72159	AC	A	4.0%	1815	725	80	33	70.8%	29.2%	Intergenic	intergenic
SEM18	72169	C	CA	7.3%	1785	900	137	104	56.8%	43.2%	Intergenic	intergenic
SEM18	75816	T	TG	2.8%	1281	3373	48	92	34.3%	65.7%	Intergenic	intergenic
SEM18	76022	CCCCCCC	A	39.9%	1520	795	398	1234	24.4%	75.6%	Intergenic	intergenic
SEM18	76047	C	CGTTGGCG	4.4%	1658	2130	73	104	41.2%	58.8%	Intergenic	intergenic
SEM18	86363	T	TC	4.1%	4428	3035	200	127	61.2%	38.8%	Intergenic	intergenic
SEM18	86773	G	GC	3.3%	3504	3700	121	128	48.6%	51.4%	Frameshift mutation	UL43
SEM18	90381	C	CA	3.0%	2655	3694	80	120	40.0%	60.0%	Intergenic	intergenic
SEM18	90434	G	GT	4.7%	3359	3308	192	144	57.1%	42.9%	Intergenic	intergenic
SEM18	100015	G	GT	36.0%	2610	2578	1612	1405	53.4%	46.6%	Intergenic	intergenic
SEM18	107399	AC	A	16.3%	1987	1614	163	683	19.3%	80.7%	Intergenic	intergenic
SEM18	109700	GC	G	4.2%	8890	3006	219	325	40.3%	59.7%	Intergenic	intergenic
SEM18	109701	C	CG	3.0%	8782	2917	137	245	35.9%	64.1%	Intergenic	intergenic
SEM18	109741	G	GC	18.0%	3917	4500	743	1251	37.3%	62.7%	Intergenic	intergenic
SEM18	110450	G	GC	28.2%	587	188	255	82	75.7%	24.3%	Intergenic	intergenic
SEM18	111898	T	TC	4.3%	4749	936	222	40	84.7%	15.3%	Intergenic	intergenic
SEM18	112108	TC	T	15.2%	1053	1614	175	370	32.1%	67.9%	Intergenic	intergenic
SEM18	112203	AC	A	34.3%	1254	1435	687	1004	40.6%	59.4%	Intergenic	intergenic
SEM18	114688	T	TC	3.1%	200	16	6	1	85.7%	14.3%	Frameshift mutation	RL2
SEM18	125324	AC	A	2.8%	3480	3143	109	86	55.9%	44.1%	Frameshift mutation	US1
SEM18	126402	CG	C	3.5%	1958	1359	72	54	57.1%	42.9%	Intergenic	intergenic
SEM18	131993	TG	T	10.0%	571	2486	57	320	15.1%	84.9%	Intergenic	intergenic
SEM18	135647	T	TC	2.2%	862	2985	62	24	72.1%	27.9%	Frameshift mutation	US8
SEM18	137498	TG	T	4.4%	2836	2162	126	114	52.5%	47.5%	Intergenic	intergenic
SEM18	137554	C	CG	6.9%	2423	2035	194	170	53.3%	46.7%	Intergenic	intergenic
SEM18	137665	G	GC	4.0%	3135	2655	161	89	64.4%	35.6%	Intergenic	intergenic
SEM18	139258	C	CATGGTGTCCCGTC	3.8%	111	1147	5	45	10.0%	90.0%	Frameshift mutation	US10
DISS29	8800	CTG	C	2.2%	594	347	11	10	52.4%	47.6%	Intergenic	intergenic
DISS29	13030	C	CGGA	8.1%	667	379	71	22	76.3%	23.7%	Inframe insertion	UL9
DISS29	15255	G	GT	22.7%	342	249	113	77	59.5%	40.5%	Intergenic	intergenic
DISS29	16564	CCCGGCG	C	2.6%	763	455	18	14	56.3%	43.8%	Disruptive inframe deletion	UL12
DISS29	20678	G	GCCC	12.5%	462	375	85	65	56.7%	43.3%	Intergenic	intergenic
DISS29	20694	C	CGG	11.3%	564	504	66	71	48.2%	51.8%	Intergenic	intergenic
DISS29	22338	AGCC	A	7.1%	741	360	64	20	76.2%	23.8%	Disruptive inframe deletion	UL17
DISS29	24089	TG	T	34.3%	274	382	217	126	63.3%	36.7%	Intergenic	intergenic
DISS29	25376	TC	T	33.3%	69	78	59	24	71.1%	28.9%	Frameshift mutation	UL15
DISS29	26515	TC	T	24.1%	296	86	95	32	74.8%	25.2%	Intergenic	intergenic
DISS29	26690	G	GAC	8.2%	443	85	37	10	78.7%	21.3%	Intergenic	intergenic
DISS29	26771	G	GTT	21.1%	432	257	178	44	80.2%	19.8%	Intergenic	intergenic
DISS29	27864	TG	T	27.5%	452	237	200	62	76.3%	23.7%	Intergenic	intergenic
DISS29	28030	CT	C	30.3%	440	356	274	73	79.0%	21.0%	Intergenic	intergenic
DISS29	28104	G	GAA	22.2%	420	311	141	127	52.6%	47.4%	Intergenic	intergenic
DISS29	32391	CA	C	25.3%	393	366	211	82	72.0%	28.0%	Intergenic	intergenic
DISS29	32464	AAAAG	A	2.2%	651	406	20	4	83.3%	16.7%	Intergenic	intergenic
DISS29	33875	TC	T	13.1%	252	387	33	68	32.7%	67.3%	Intergenic	intergenic
DISS29	37652	C	CGGGCGGCCCCGG	5.9%	775	423	56	20	73.7%	26.3%	Inframe insertion	UL22
DISS29	38277	TGGGG	T	25.4%	338	203	196	66	74.8%	25.2%	Intergenic	intergenic
DISS29	40478	GT	G	12.9%	248	184	51	21	70.8%	29.2%	Intergenic	intergenic
DISS29	40665	T	TC	31.3%	341	215	235	28	89.4%	10.6%	Intergenic	intergenic

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	INDEL Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	43628	GTCT	G	27.9%	528	379	273	79	77.6%	22.4%	Inframe deletion	UL26
DISS29	44673	TG	T	34.1%	94	93	62	42	59.6%	40.4%	Intergenic	intergenic
DISS29	44927	C	CGGGGTTGGTTT	25.6%	197	122	100	22	82.0%	18.0%	Intergenic	intergenic
DISS29	44999	CCA	C	34.0%	245	112	140	44	76.1%	23.9%	Intergenic	intergenic
DISS29	45090	CCCCTT	C	3.6%	225	278	9	10	47.4%	52.6%	Intergenic	intergenic
DISS29	50207	T	TG	17.8%	297	371	140	23	85.9%	14.1%	Intergenic	intergenic
DISS29	50284	TCGCC	T	2.1%	93	417	7	4	63.6%	36.4%	Intergenic	intergenic
DISS29	50350	TC	T	35.2%	50	200	32	106	23.2%	76.8%	Intergenic	intergenic
DISS29	50379	AC	A	21.2%	21	177	20	48	29.4%	70.6%	Intergenic	intergenic
DISS29	54263	C	CTGGGGG	16.7%	689	268	163	35	82.3%	17.7%	Intergenic	intergenic
DISS29	54353	C	CG	12.0%	898	415	135	53	71.8%	28.2%	Intergenic	intergenic
DISS29	54519	C	CGCG	3.2%	1189	640	8	53	13.1%	86.9%	Intergenic	intergenic
DISS29	54726	TG	T	16.5%	337	145	20	90	18.2%	81.8%	Intergenic	intergenic
DISS29	54732	G	GCTGGTGA	34.5%	220	227	197	43	82.1%	17.9%	Intergenic	intergenic
DISS29	54761	GGGGT	G	23.7%	307	409	151	74	67.1%	32.9%	Intergenic	intergenic
DISS29	56741	AGGC	A	52.3%	46	277	275	80	77.5%	22.5%	Inframe deletion	UL30
DISS29	56825	ACGAGGC	A	34.6%	199	302	176	90	66.2%	33.8%	Inframe deletion	UL30
DISS29	60354	GCCCGCC	G	19.2%	522	319	144	56	72.0%	28.0%	Disruptive inframe deletion	UL32
DISS29	62263	GGGCGGT	G	7.0%	456	397	44	20	68.8%	31.3%	Inframe deletion	UL34
DISS29	62972	G	GC	3.1%	379	405	17	8	68.0%	32.0%	Intergenic	intergenic
DISS29	63045	T	TG	10.2%	100	267	19	24	44.2%	55.8%	Intergenic	intergenic
DISS29	66656	G	GGGGCGC	17.6%	518	378	124	69	64.2%	35.8%	Inframe insertion	UL36
DISS29	76228	G	GT	4.5%	184	169	9	8	52.9%	47.1%	Intergenic	intergenic
DISS29	76433	CCCA	C	9.5%	560	270	20	68	22.7%	77.3%	Intergenic	intergenic
DISS29	77933	CT	C	3.9%	317	195	4	17	19.0%	81.0%	Intergenic	intergenic
DISS29	77945	CCCT	C	5.1%	246	195	3	21	12.5%	87.5%	Intergenic	intergenic
DISS29	78804	TCGGGCCC	C	8.6%	452	408	55	26	67.9%	32.1%	Inframe deletion	UL39
DISS29	86708	TC	T	22.7%	516	316	200	57	77.8%	22.2%	Intergenic	intergenic
DISS29	86771	A	AC	37.9%	350	332	250	170	59.5%	40.5%	Intergenic	intergenic
DISS29	87117	G	GC	2.1%	562	452	11	11	50.0%	50.0%	Frameshift mutation	UL43
DISS29	90642	C	CCCCCTCCCCA	20.7%	326	369	134	71	65.4%	34.6%	Intergenic	intergenic
DISS29	90701	TC	T	21.3%	329	370	130	81	61.6%	38.4%	Intergenic	intergenic
DISS29	90709	C	CA	4.8%	456	463	26	22	54.2%	45.8%	Intergenic	intergenic
DISS29	90761	G	GT	4.8%	464	442	21	28	42.9%	57.1%	Intergenic	intergenic
DISS29	90792	GA	G	8.3%	492	401	40	41	49.4%	50.6%	Intergenic	intergenic
DISS29	90849	ATT	A	35.9%	343	238	200	142	58.5%	41.5%	Intergenic	intergenic
DISS29	91255	A	AGGC	9.0%	275	390	46	20	69.7%	30.3%	Inframe insertion	UL46
DISS29	95264	A	AG	24.4%	260	84	95	18	84.1%	15.9%	Intergenic	intergenic
DISS29	95537	A	AC	34.6%	360	113	191	64	74.9%	25.1%	Intergenic	intergenic
DISS29	95686	TG	T	39.1%	172	276	184	134	57.9%	42.1%	Intergenic	intergenic
DISS29	100706	GTT	G	21.4%	379	356	174	66	72.5%	27.5%	Intergenic	intergenic
DISS29	108088	A	AC	3.3%	259	320	9	12	42.9%	57.1%	Frameshift mutation	UL54
DISS29	108933	AC	A	13.8%	146	42	6	27	18.2%	81.8%	Intergenic	intergenic
DISS29	110754	G	GC	6.7%	31	79	5	3	62.5%	37.5%	Intergenic	intergenic
DISS29	110759	G	GT	13.3%	25	63	5	9	35.7%	64.3%	Intergenic	intergenic
DISS29	110967	GC	G	5.8%	537	67	30	9	76.9%	23.1%	Intergenic	intergenic
DISS29	110968	C	CG	2.3%	527	65	7	8	46.7%	53.3%	Intergenic	intergenic
DISS29	111007	GCC	G	5.4%	311	129	17	11	60.7%	39.3%	Intergenic	intergenic
DISS29	111013	CCCCCA	C	8.9%	337	119	14	32	30.4%	69.6%	Intergenic	intergenic
DISS29	111141	C	CG	9.2%	98	126	15	8	65.2%	34.8%	Intergenic	intergenic
DISS29	111181	AG	A	17.4%	64	289	26	54	32.5%	67.5%	Intergenic	intergenic
DISS29	111309	GC	G	15.3%	56	104	11	24	31.4%	68.6%	Intergenic	intergenic
DISS29	111723	C	CG	7.8%	253	73	21	9	70.0%	30.0%	Intergenic	intergenic
DISS29	111731	C	CCGCCGCCA	4.3%	290	89	10	7	58.8%	41.2%	Intergenic	intergenic
DISS29	111749	CG	C	2.1%	216	107	4	3	57.1%	42.9%	Intergenic	intergenic
DISS29	111762	GCC	G	15.7%	143	102	42	10	80.8%	19.2%	Intergenic	intergenic
DISS29	111768	CCCCCA	C	18.8%	159	106	39	24	61.9%	38.1%	Intergenic	intergenic
DISS29	111896	C	CG	38.5%	129	96	128	21	85.9%	14.1%	Intergenic	intergenic
DISS29	111936	AG	A	26.2%	179	80	94	32	74.6%	25.4%	Intergenic	intergenic
DISS29	112062	G	GC	9.6%	264	115	27	18	60.0%	40.0%	Intergenic	intergenic
DISS29	112373	GT	G	2.4%	633	9	14	2	87.5%	12.5%	Intergenic	intergenic
DISS29	112487	G	GTT	8.8%	903	111	89	15	85.6%	14.4%	Intergenic	intergenic
DISS29	112753	CT	C	8.9%	114	841	15	79	16.0%	84.0%	Intergenic	intergenic
DISS29	113179	TTCTC	T	18.5%	110	11	21	10	67.7%	32.3%	Intergenic	intergenic
DISS29	113310	CGG	C	17.7%	146	35	52	12	81.3%	18.8%	Intergenic	intergenic
DISS29	113428	AGG	A	29.0%	82	85	70	31	69.3%	30.7%	Intergenic	intergenic
DISS29	113470	G	GGGAAAA	22.6%	89	16	19	28	40.4%	59.6%	Intergenic	intergenic
DISS29	113523	G	GGGGGGGC	4.0%	299	36	13	2	86.7%	13.3%	Intergenic	intergenic
DISS29	113734	A	AC	25.8%	260	145	92	52	63.9%	36.1%	Intergenic	intergenic
DISS29	113777	TC	T	8.2%	58	216	6	20	23.1%	76.9%	Intergenic	intergenic
DISS29	113854	A	AC	10.4%	28	196	6	22	21.4%	78.6%	Intergenic	intergenic
DISS29	114026	A	AC	13.2%	385	101	54	25	68.4%	31.6%	Intergenic	intergenic
DISS29	114119	G	GGTA	30.5%	545	120	224	75	74.9%	25.1%	Intergenic	intergenic
DISS29	114151	C	CG	51.2%	147	196	161	217	42.6%	57.4%	Intergenic	intergenic
DISS29	114628	TC	T	45.5%	81	406	95	314	23.2%	76.8%	Intergenic	intergenic
DISS29	114963	TCC	T	31.3%	36	291	25	144	14.8%	85.2%	Intergenic	intergenic
DISS29	115074	G	GT	18.7%	687	330	197	94	67.7%	32.3%	Intergenic	intergenic
DISS29	115157	T	TC	4.0%	1053	311	13	51	20.3%	79.7%	Intergenic	intergenic
DISS29	115905	C	CGGAGGA	2.3%	200	772	12	11	52.2%	47.8%	Inframe insertion	RL2
DISS29	115929	A	AGGCGGC	11.1%	102	590	38	49	43.7%	56.3%	Inframe insertion	RL2
DISS29	117790	C	CG	8.2%	146	1175	38	81	31.9%	68.1%	Intergenic	intergenic
DISS29	117984	C	CAGGG	7.9%	927	807	99	51	66.0%	34.0%	Intergenic	intergenic
DISS29	118296	GTC	G	27.5%	914	731	470	156	75.1%	24.9%	Intergenic	intergenic
DISS29	119278	TGGGCCC	T	18.5%	149	61	41	7	85.4%	14.6%	Disruptive inframe deletion	RL1
DISS29	120031	C	CGG	8.6%	79	24	8	2	80.0%	20.0%	Frameshift mutation	RL1
DISS29	120256	A	AC	36.2%	146	63	93	32	74.4%	25.6%	Intergenic	intergenic
DISS29	120303	GT	G	17.5%	145	53	17	25	40.5%	59.5%	Intergenic	intergenic
DISS29	120590	G	GC	32.3%	147	24	60	23	72.3%	27.7%	Intergenic	intergenic
DISS29	120709	G	GGCC	6.6%	44	130	10	4	71.4%	28.6%	Intergenic	intergenic
DISS29	122818	G	GGCGC	13.8%	95	11	15	2	88.2%	11.8%	Frameshift mutation	RS1
DISS29	122821	C	CAG	31.0%	44	9	31	4	88.6%	11.4%	Frameshift mutation	RS1
DISS29	122834	G	GAAGC	60.5%	76	7	131	16	89.1%	10.9%	Frameshift mutation	RS1

Supplemental Table S3. Minor variant SNPs and INDELs in neonatal HSV-2 genomes (two Excel tabs)

Isolate	INDEL Position in the genome	Major allele	Minor allele	Minor allele frequency	Reads supporting major allele on forward strand	Reads supporting major allele on reverse strand	Reads supporting minor allele on forward strand	Reads supporting minor allele on reverse strand	Percent reads supporting minor allele on forward strand	Percent reads supporting minor allele on reverse strand	Type of variation	Gene name
DISS29	123907	GTCA	G	16.0%	760	180	131	49	72.8%	27.2%	Disruptive inframe deletion	RS1
DISS29	123910	A	ATCGTCG	8.5%	692	101	27	54	33.3%	66.7%	Disruptive inframe insertion	RS1
DISS29	124365	G	GCGTCGT	6.1%	104	509	19	21	47.5%	52.5%	Inframe insertion	RS1
DISS29	124857	ATCGTCG	A	43.3%	374	108	103	284	26.6%	73.4%	Intergenic	intergenic
DISS29	125110	GCAT	G	38.9%	300	147	164	121	57.5%	42.5%	Intergenic	intergenic
DISS29	125321	G	GCGTCGT	7.8%	829	171	76	10	88.4%	11.6%	Intergenic	intergenic
DISS29	125386	C	CGGGG	10.2%	678	160	91	10	90.1%	9.9%	Intergenic	intergenic
DISS29	125392	G	GGGGGTCTTCT	20.9%	505	86	146	55	72.6%	27.4%	Intergenic	intergenic
DISS29	125442	G	GTC	7.7%	362	581	14	65	17.7%	82.3%	Intergenic	intergenic
DISS29	125595	CG	C	25.4%	217	535	144	113	56.0%	44.0%	Intergenic	intergenic
DISS29	126727	CG	C	6.4%	326	35	19	6	76.0%	24.0%	Intergenic	intergenic
DISS29	126730	G	GAC	3.7%	332	76	14	2	87.5%	12.5%	Intergenic	intergenic
DISS29	126734	A	ACGGGACCC	27.9%	187	60	110	19	85.3%	14.7%	Intergenic	intergenic
DISS29	127164	AC	A	2.4%	618	413	16	9	64.0%	36.0%	Frameshift mutation	US1
DISS29	128177	CAAA	C	18.3%	359	112	94	34	73.4%	26.6%	Intergenic	intergenic
DISS29	128245	C	CG	24.9%	206	209	87	75	53.7%	46.3%	Intergenic	intergenic
DISS29	128514	C	CGCCGGG	21.6%	251	159	86	29	74.8%	25.2%	Inframe insertion	US2
DISS29	130884	AGG	A	31.1%	352	411	247	123	66.8%	33.2%	Intergenic	intergenic
DISS29	132242	CGCG	C	32.5%	150	346	141	98	59.0%	41.0%	Inframe deletion	US4
DISS29	133078	T	TG	30.2%	245	244	157	65	70.7%	29.3%	Intergenic	intergenic
DISS29	136185	GCCCGCA	G	30.3%	163	267	67	122	35.4%	64.6%	Inframe deletion	US7
DISS29	136658	T	TC	23.5%	333	378	101	120	45.7%	54.3%	Intergenic	intergenic
DISS29	136767	C	CG	9.2%	417	315	55	33	62.5%	37.5%	Intergenic	intergenic
DISS29	139344	T	TG	17.2%	342	373	114	58	66.3%	33.7%	Intergenic	intergenic
DISS29	139355	GA	G	9.4%	467	402	43	47	47.8%	52.2%	Intergenic	intergenic
DISS29	139391	C	CG	11.8%	451	392	84	29	74.3%	25.7%	Intergenic	intergenic
DISS29	139397	C	CG	5.4%	466	395	30	24	55.6%	44.4%	Intergenic	intergenic
DISS29	139558	G	GCCCC	4.3%	370	312	23	12	65.7%	34.3%	Intergenic	intergenic
DISS29	141159	C	\CATGGTGTCCCGTC	3.0%	58	172	2	5	28.6%	71.4%	Frameshift mutation	US10
DISS29	141161	A	CATGGTGTCCCGTC	8.7%	39	97	2	18	10.0%	90.0%	Frameshift mutation	US10
DISS29	141162	G	GACATGGTGT	4.8%	30	103	3	8	27.3%	72.7%	Inframe insertion	US10