Supplementary Material

Table S1. Predicted size (bp) of the PCR products using the different oligonucleotides combination for both WT and mutant template genome for all the assayed genes.

Gene	WT ab[1]	Mutant ab[1]	Mutant ac[2]	Mutant db[3]
UL2	520	1961	1067	1285
UL5	928	2151	1079	1363
UL6	1342	2211	1226	1276
UL7	1114	2169	1141	1319
UL8	831	2186	1206	1291
UL9	1157	2194	1179	1306
UL44	1903	2325	1454	1162
UL121	893	2125	1179	1237
US7	1030	2081	1070	1302
US9	1192	2172	1050	1413
US13	1150	2668	1130	1249
US14	1387	2178	1119	1350
US16	1549	2343	1425	1209
US17	1410	2252	1239	1304
US18	1362	2261	1208	1344
US20	1319	2278	1189	1380
US21	1242	2246	1270	1267
US30	1974	2648	1310	1629
pp65	1905	1949	1044	1196
IE1	2132	2096	1018	1369
gO	1796	2119	1131	1279
gM	1410	2015	1126	1180

[1] Amplification using the primers upstream and downstream (ab) of the target gene using the wild-type BAC DNA (WT) as a template.
[2] Amplification using the primer upstream of the deleted gene and an internal Kan primer (ac).
[3] Amplification using the internal Kan primer and a primer downstream of the deleted gene (db).

Figure S1. Representative step-by-step process to validate UL6 deletion mutant. **(A)** DNA of the WT BAC and the BAC obtained from 8 colonies were digested with *Eco*RI and *Bam*HI enzymes and the restriction patterns obtained are shown. Digestion products were separated by electrophoresis on a 1% agarose gel. Lane 1: wild-type BAC DNA (WT). Lane 2-9: BAC DNA obtained from the 8 tested colonies for ΔUL6. **(B)** To test the insertion of the Kan cassette in the UL6 ORF, the BACs obtained from the 8 tested colonies were used for PCR amplification using the upstream and dowstream (ab) primers. Only clones 3 and 4 (highlighted in green) were positive for the insertion, while the band obtained from the other 6 clones had the size expected for WT. **(C)** A second PCR amplification was performed using clones 3 and 4 using the internal Kan primer and a primer downstream or upstream of the deleted gene (db or ac, respectively). PCR products were sequenced. For all the experiments 1 kb DNA molecular weight marker was used as a ladder.

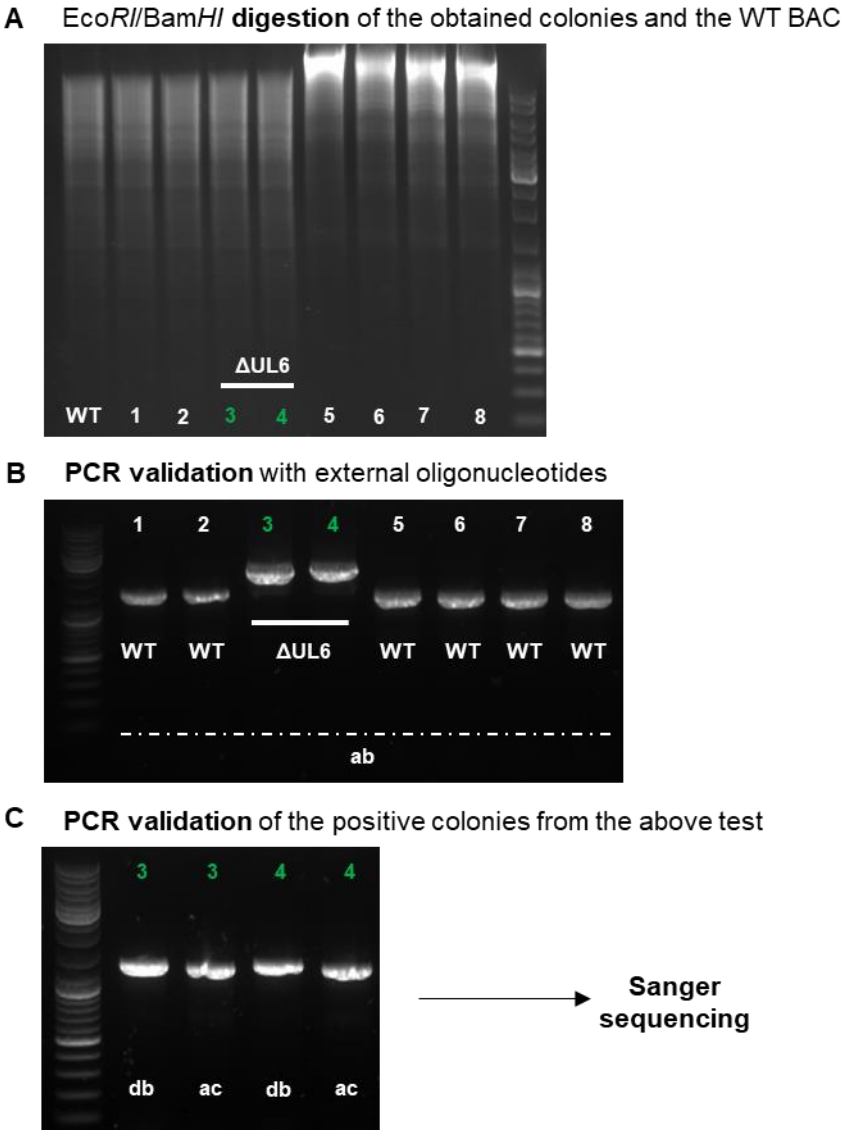

Figure S2. Representative images of PCR amplification products obtained to confirm the gene deletion of the indicated ORF. PCR products were separated by electrophoresis on a 1.5% agarose gel. Lane 1: 1 kb DNA molecular weight marker used as a ladder. Lane 2 and 3: PCR amplification products obtained using the primers upstream and downstream (ab) of the target gene using as a template either the wild-type BAC DNA (WT) or the indicated deletion mutant BAC DNA. Lane 4: PCR amplification product obtained using the internal Kan primer and a primer downstream of the deleted gene (db). Lane 5: PCR amplification product obtained using the primer upstream of the deleted gene and an internal Kan primer (ac).