Abstract: Recombinant plasmids containing African swine fever virus (ASFV) DNA fragments covering all the virus genome were transfected into infected cells in order to detect viral origins of DNA replication. Plasmid replication was monitored by sensitivity to Mbol, which cleaves only replicated, unmethylated DNA, and resistance to Dpnl, which cleaves only the same methylated sequence. All the recombinants replicated to a similar extent, indicating that ASFV does not use a preferred origin for DNA replication. Circular plasmids without viral inserts were also replicated, but linearized plasmids or lambda bacteriophage DNA were not replicated. Replicated plasmid DNA began to accumulate with a time course similar to viral DNA, starting between 6 and 12 hr p.i. and increasing steadily for about 18 hr. This apparent dependence on Viral functions was confirmed by the sensitivity of plasmid replication to phosphonoacetic acid and resistance to aphidicolin and by the reduction of replication in cells infected with a mutant defective in DNA replication. Replicated plasmid DNA was present as unit length circles and as large dimension forms, probably head-to-tail concatemers. The results of two-dimensional electrophoresis (neutral/alkaline) favor a rolling-circle mechanism for plasmid DNA replication. (C) Press Academic Press, Inc.