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Generation of modified pestiviruses by targeted recombination

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Infectious cDNA clones are a prerequisite for directed genetic manipulation of pestivirus RNA genomes. We have developed a novel strategy to facilitate manipulation and rescue of modified pestiviruses from infectious cDNA clones based on bacterial artificial chromosomes (BACs). The strategy involves targeted modification of viral cDNA genomes, cloned within BACs, by Red/ET recombination-mediated mutagenesis in E.coli DH10B cells. Using recombination-mediated mutagenesis for the targeted design, the work can be expedited and focused in principal on any sequence within the viral genome and hence is not limited to the use of internal restriction sites. Rescue of modified pestiviruses can be obtained by electroporation of cell cultures with full-length RNA transcripts *in vitro* transcribed from the recombined BAC clones. We have used this approach to generate a series of new pestivirus BACs modified within different genomic regions and infectious pestiviruses have been rescued from several of these new constructs, demonstrating that recombination-mediated mutagenesis of pestivirus BACs provides a useful tool for expediting the construction of recombinant pestiviruses.