CLAIMS

1. A bacmid comprising:
a bacterial replicon;
a selectable marker sequence;
a first reporter gene comprising a first preferential target site for the insertion of a transposon;
a second reporter gene operably linked to a baculovirus-inducible promoter; and
a second preferential target site capable of mediating a site-specific recombination event.

2. The bacmid of claim 1, wherein the bacterial replicon is a low-copy number replicon.

3. The bacmid of claim 2, wherein the low-copy number replicon is a mini-F replicon.

4. The bacmid of any preceding claim, wherein the selectable marker sequence comprises an antibiotic resistance gene.

5. The bacmid of claim 4, wherein the antibiotic resistance gene is a kanamycin resistance gene.

6. The bacmid of any preceding claim, wherein the first reporter gene encodes an enzyme capable of metabolizing a chromogenic substrate.

7. The bacmid of claim 6, wherein the enzyme is LacZα or functional portion thereof.

8. The bacmid of claim 6 or 7, wherein the chromogenic substrate is Blue-gal or X-gal.

9. The bacmid of any preceding claim, wherein the first preferential target site for the insertion of a transposon does not disrupt the reading frame of the first reporter gene.

10. The bacmid of any preceding claim, wherein the first preferential target site is an attachment site for a bacterial transposon.

11. The bacmid of any preceding claim, wherein the first preferential target site is an attachment site for a T7 transposon.

12. The bacmid of claim 11, wherein the transposon is a T7 transposon.

13. The bacmid of any preceding claim, wherein the second reporter gene encodes a fluorescent protein.

14. The bacmid of claim 13, wherein the fluorescent protein is a red fluorescent protein.

15. The bacmid of any preceding claim, wherein the baculovirus-inducible promoter is a 39K promoter.

16. The bacmid of any preceding claim, wherein the second preferential target site comprises a LoxP site or a variant thereof.

17. The bacmid of any preceding claim, wherein the site-specific recombination event is mediated by a Cre recombinase.

18. A bacmid comprising:
a mini-F replicon;
an antibiotic resistance gene;
a LacZα gene or functional portion thereof comprising an attachment site for a T7 transposon;
a gene encoding a fluorescent protein operably linked to a baculovirus-inducible promoter; and
a LoxP site or a variant thereof.

19. A recombinant bacmid comprising:
a bacterial replicon;
a first selectable marker sequence;
a heterologous sequence inserted into a first reporter gene, wherein the inserted heterologous sequence disrupts the reading frame of the first reporter gene;
a second reporter gene operably linked to a baculovirus-inducible promoter; and
a preferential target site capable of mediating a site-specific recombination event.

20. The recombinant bacmid of claim 19, wherein the heterologous sequence comprises a heterologous gene.

21. The recombinant bacmid of claim 19 or 20, wherein the heterologous gene comprises a sequence encoding a protein.

22. The recombinant bacmid of claim 20 or 21, wherein the heterologous sequence comprises an expression control sequence.

23. The recombinant bacmid of claim 22, wherein the expression control sequence is operably linked to the sequence encoding a protein.

24. The recombinant bacmid of claim 22 or 23, wherein the expression control sequence comprises a baculovirus promoter.

25. The recombinant bacmid of claim 24, wherein the baculovirus promoter is an immediate early, an early, a late, or a very late gene promoter.

26. The recombinant bacmid of claim 24 or 25, wherein the baculovirus promoter is selected from the group consisting of a polyhedrin promoter, an immediate-early1 promoter, and an immediate-early2 promoter.

27. The recombinant bacmid of any one of claims 22-26, wherein the expression control sequence comprises a polyadenylation signal.

28. The recombinant bacmid of any one of claims 21-27, wherein the protein is a Rep protein isolated from a genome of a member of the viral family Parvoviridae.

29. The recombinant bacmid of any one of claims 21-28, wherein the protein is a parvoviral Rep protein.

30. The recombinant bacmid of claim 29, wherein the parvoviral Rep protein is selected from the group consisting of a B19 Rep, an AAV2 Rep, a HBoV1 Rep, and a GPV Rep.

31. The recombinant bacmid of any one of claims 19-30, wherein the heterologous sequence comprises a second selectable marker sequence.

32. The recombinant bacmid of claim 31, wherein the second selectable marker sequence comprises a gentamycin resistance gene.

33. The recombinant bacmid of any one of claims 19-32, wherein the bacterial replicon is mini-F replicon.

34. The recombinant bacmid of any one of claims 19-33, wherein the first selectable marker sequence comprises a kanamycin resistance gene.

35. The recombinant bacmid of any one of claims 19-34, wherein the first reporter gene encodes LacZα or functional portion thereof.

36. The recombinant bacmid of any one of claims 19-35, wherein the second reporter gene encodes a red fluorescent protein.

37. The recombinant bacmid of any one of claims 19-36, wherein the baculovirus-inducible promoter is a 39K promoter.

38. The recombinant bacmid of any one of claims 19-37, wherein the preferential target site comprises a LoxP site or a variant thereof.

39. The recombinant bacmid of any one of claims 19-38, wherein the site-specific recombination event is mediated by a Cre recombinase.

40. A recombinant bacmid comprising:
a heterologous sequence inserted into a mini-attTn7 site, wherein the heterologous sequence encodes a Rep, and wherein the inserted Rep disrupts the reading frame of the LacZα gene or functional portion thereof; and
a LoxP site or a variant thereof.

41. A recombinant bacmid comprising:
a bacterial replicon;
a first antibiotic resistance gene;

a heterologous sequence inserted into a mini-attTn7 site, wherein the heterologous sequence encodes a Rep, and wherein the inserted Rep disrupts the reading frame of the LacZα gene or functional portion thereof;
a gene encoding a fluorescent protein operably linked to a baculovirus-inducible promoter; and
a LoxP site or a variant thereof.

42. A recombinant bacmid comprising:
a bacterial replicon;
a first antibiotic resistance gene;
a heterologous sequence inserted into a mini-attTn7 site, wherein the heterologous sequence encodes a B19 Rep, and wherein the inserted B19 Rep disrupts the reading frame of the LacZα gene or functional portion thereof;
a gene encoding a fluorescent protein operably linked to a baculovirus-inducible promoter; and
a LoxP site or a variant thereof.

43. A recombinant bacmid comprising:
a bacterial replicon;
a first antibiotic resistance gene;
a heterologous sequence inserted into a mini-attTn7 site, wherein the heterologous sequence encodes a GPV Rep, and wherein the inserted GPV Rep disrupts the reading frame of the LacZα gene or functional portion thereof;
a gene encoding a fluorescent protein operably linked to a baculovirus-inducible promoter; and
a LoxP site or a variant thereof.

44. A recombinant bacmid comprising:
a bacterial replicon;
a first antibiotic resistance gene;
a heterologous sequence inserted into a mini-attTn7 site, wherein the heterologous sequence encodes an AAV2 Rep, and wherein the inserted AAV2 Rep disrupts the reading frame of the LacZα gene or functional portion thereof;
a gene encoding a fluorescent protein operably linked to a baculovirus-inducible promoter; and
a LoxP site or a variant thereof.

45. A nucleic acid vector comprising:

a first origin of replication for propagating the nucleic acid vector in a first bacterial strain, wherein the first origin of replication is a conditional origin of replication;
a second origin of replication for propagating the nucleic acid vector in a second bacterial strain;
a multiple cloning site for the insertion of a heterologous sequence;
a selectable marker sequence;
a reporter gene; and
a preferential target site capable of mediating a site-specific recombination event.

46. The vector of claim 45, wherein the first origin of replication is conditional upon the presence of a pi protein.

47. The vector of claim 45 or 46, wherein the first origin of replication is R6Kγ.

48. The vector of any one of claims 45-47, wherein the first bacterial strain comprises a pi protein.

49. The vector of any one of claims 45-48, wherein the second origin of replication is pUC57.

50. The vector of any one of claims 45-49, wherein the selectable marker sequence comprises an antibiotic resistance gene.

51. The vector of claim 50, wherein the antibiotic resistance gene is an ampicillin resistance gene.

52. The vector of any one of claims 45-51, wherein the reporter gene encodes a fluorescent protein.

53. The vector of claim 52, wherein the fluorescent protein is a green fluorescent protein.

54. The vector of any one of claims 45-53, wherein the preferential target site comprises a LoxP site or a variant thereof.

55. The vector of any one of claims 45-54, wherein the site-specific recombination event is mediated by a Cre recombinase.

56. A nucleic acid vector comprising:
a first origin of replication for propagating the nucleic acid vector in a first bacterial strain, wherein the first origin of replication is a conditional origin of replication;
a second origin of replication for propagating the nucleic acid vector in a second bacterial strain;
a heterologous sequence;
a selectable marker sequence;
a reporter gene; and
a preferential target site capable of mediating a site-specific recombination event.

57. The vector of claim 56, wherein the heterologous sequence comprises a heterologous gene.

58. The vector of claim 57, wherein the heterologous gene comprises a sequence encoding a protein.

59. The vector of claim 57 or 58, wherein the heterologous sequence comprises an expression control sequence.

60. The vector of claim 59, wherein the expression control sequence is operably linked to the sequence encoding a protein.

61. The vector of claim 59 or 60, wherein the expression control sequence comprises a tissue-specific promoter.

62. The vector of claim 61, wherein the tissue-specific promoter is a tristetraprolin (TTP) or a murine transthyretin (mTTR) promoter.

63. The vector of any one of claims 59-62, wherein the expression control sequence comprises a polyadenylation signal.

64. The vector of claim 63, wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal.

65. The vector of any one of claims 59-64, wherein the expression control sequence comprises a post-transcriptional regulatory element.

66. The vector of claim 65, wherein the post-transcriptional regulatory element is a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

67. The vector of any one of claims 58-66, wherein the protein is a therapeutic protein.

68. The vector of claim 67, wherein the therapeutic protein is a clotting factor.

69. The vector of claim 66, wherein the clotting factor is Factor VIII (FVIII).

70. The vector of claim 68 or 69, wherein the clotting factor is FVIII-XTEN.

71. The vector of any one of claims 56-70, wherein the heterologous sequence comprises a 5' inverted terminal repeat (ITR).

72. The vector of any one of claims 56-71, wherein the heterologous sequence comprises a 3' inverted terminal repeat (ITR).

73. The vector of claim 71 or 72, wherein the 5' ITR and the 3' ITR are derived from a parvovirus.

74. The vector of claim 73, wherein the parvovirus is selected from the group consisting of B19, GPV, HBoV1, and AAV2.

75. The vector of any one of claims 71-74, wherein the 5' ITR is a wild-type or variant 5' ITR derived from B19.

76. The vector of any one of claims 71-74, wherein the 5' ITR is a wild-type or variant 5' ITR derived from GPV.

77. The vector of any one of claims 71-74, wherein the 5' ITR is a wild-type or variant 5' ITR derived from AAV2.

78. The vector of any one of claims 71-74, wherein the 5’ ITR is a wild-type 5’ ITR derived from HBoV1.

79. The vector of any one of claims 75-77, wherein the variant 5’ ITR is a truncated 5’ ITR.

80. The vector of any one of claims 71-79, wherein the 3’ ITR is a wild-type or variant 3’ ITR derived from B19.

81. The vector of any one of claims 71-78, wherein the 3’ ITR is a wild-type or variant 3’ ITR derived from GPV.

82. The vector of any one of claims 71-78, wherein the 3’ ITR is a wild-type or variant 3’ ITR derived from AAV2.

83. The vector of any one of claims 71-78, wherein the 3’ ITR is a wild-type 3’ ITR derived from HBoV1.

84. The vector of any one of claims 80-82, wherein the variant 3’ ITR is a truncated 3’ ITR.

85. The vector of any one of claims 56-76, wherein the first origin of replication is conditional upon the presence of a pi protein.

86. The vector of claim 85, wherein the first origin of replication is R6Kγ.

87. The vector of claim 85 or 86, wherein the first bacterial strain comprises a pi protein.

88. The vector of any one of claims 56-87, wherein the second origin of replication is pUC57.

89. The vector of any one of claims 56-88, wherein the selectable marker sequence comprises an antibiotic resistance gene.

90. The vector of claim 89, wherein the antibiotic resistance gene is an ampicillin resistance gene.

91. The vector of any one of claims 56-90, wherein the reporter gene encodes a fluorescent protein.

92. The vector of claim 91, wherein the fluorescent protein is a green fluorescent protein.

93. The vector of any one of claims 56-92, wherein the preferential target site comprises a LoxP site or a variant thereof.

94. The vector of any one of claims 56-93, wherein the site-specific recombination event is mediated by a Cre recombinase.

95. A recombinant bacmid comprising:
a first heterologous sequence inserted into a first reporter gene, wherein the inserted heterologous sequence disrupts the reading frame of the first reporter gene;
a first preferential target site capable of mediating a site-specific recombination event;
a multiple cloning site comprising a second heterologous sequence; and
a second preferential target site capable of mediating a site-specific recombination event.

96. The recombinant bacmid of claim 95, wherein the first heterologous sequence comprises a first heterologous gene.

97. The recombinant bacmid of claim 96, wherein the first heterologous sequence comprises an expression control sequence operably linked to a sequence encoding a protein.

98. The recombinant bacmid of claim 97, wherein the expression control sequence comprises a baculovirus promoter.

99. The recombinant bacmid of claim 98, wherein the baculovirus promoter is an immediate early, an early, a late, or a very late promoter.

100. The recombinant bacmid of claim 99, wherein the baculovirus promoter is selected from the group consisting of a polyhedrin promoter, an immediate-early1 promoter, and an immediate-early2 promoter.

101. The recombinant bacmid of any one of claims 97-100, wherein the protein is a Rep protein isolated from a genome of a member of the viral family Parvoviridae.

102. The recombinant bacmid of any one of claims 97-101, wherein the protein is a parvoviral Rep protein.

103. The recombinant bacmid of claim 102, wherein the parvoviral Rep protein is selected from the group consisting of a B19 Rep, an AAV2 Rep, a HBoV1 Rep, and a GPV Rep.

104. The recombinant bacmid of any one of claims 95-103, wherein the second heterologous sequence comprises a second heterologous gene.

105. The recombinant bacmid of claim 104, wherein the second heterologous sequence comprises a expression control sequence operably linked to a sequence encoding a protein.

106. The recombinant bacmid of claim 105, wherein the expression control sequence comprises a tissue-specific promoter, a polyadenylation signal, and/or a post-transcriptional regulatory element.

107. The recombinant bacmid of claim 106, wherein the tissue-specific promoter is a tristetraprolin (TTP) or a murine transthyretin (mTTR) promoter.

108. The recombinant bacmid of claim 107, wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal.

109. The recombinant bacmid of claim 107, wherein the post-transcriptional regulatory element is a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

110. The recombinant bacmid of any one of claims 105-109, wherein the protein is a therapeutic protein.

111. The recombinant bacmid of claim 110, wherein the therapeutic protein is a clotting factor.

112. The recombinant bacmid of claim 111, wherein the clotting factor is Factor VIII (FVIII).

113. The recombinant bacmid of claim 111 or 112, wherein the clotting factor is FVIII-XTEN.

114. The recombinant bacmid of any one of claims 95-113, wherein the second heterologous sequence comprises a 5' inverted terminal repeat (ITR).

115. The recombinant bacmid of any one of claims 95-114, wherein the second heterologous sequence comprises a 3' inverted terminal repeat (ITR).

116. The recombinant bacmid of claim 115, wherein the 5' ITR is derived from a genome of a first member of the viral family Parvoviridae, and the 3' ITR are derived from a genome of a second member of the viral family Parvoviridae.

117. The recombinant bacmid of claim 116, wherein the first and the second members are the same.

118. The recombinant bacmid of claim 116, wherein the first and the second members are different.

119. The recombinant bacmid of any one of claims 114-118, wherein the 5' ITR and the 3' ITR are derived from a parvovirus selected from the group consisting of B19, GPV, HBoV1, and AAV2.

120. The recombinant bacmid of claim 119, wherein the 5' ITR is a wild-type or truncated 5' ITR derived from B19.

121. The recombinant bacmid of claim 119, wherein the 5' ITR is a wild-type or truncated 5' ITR derived from GPV.

122. The recombinant bacmid of claim 119, wherein the 5' ITR is a wild-type or truncated 5' ITR derived from AAV2.

123. The recombinant bacmid of claim 119, wherein the 5' ITR is a wild-type 5' ITR derived from HBoV1.

124. The recombinant bacmid of claim 119, wherein the 3' ITR is a wild-type or truncated 3' ITR derived from B19.

125. The recombinant bacmid of claim 119, wherein the 3' ITR is a wild-type or truncated 3' ITR derived from GPV.

126. The recombinant bacmid of claim 119, wherein the 3' ITR is a wild-type or truncated 3' ITR derived from AAV2.

127. The recombinant bacmid of claim 119, wherein the 3' ITR is a wild-type 3' ITR derived from HBoV1.

128. The recombinant bacmid of any one of claims 95-126, wherein the recombinant bacmid further comprises a bacterial replicon.

129. The recombinant bacmid of claim 128, wherein the bacterial replicon is a mini-F replicon.

130. The recombinant bacmid of any one of claims 95-129, wherein the recombinant bacmid further comprises one or more selectable marker sequences.

131. The recombinant bacmid of claim 130, wherein the one or more selectable marker sequences comprise one or more antibiotic resistance genes.

132. The recombinant bacmid of claim 131, wherein the one or more antibiotic resistance genes are selected from the group consisting of an ampicillin resistance gene, a kanamycin resistance gene, and a gentamycin resistance gene.

133. The recombinant bacmid of any one of claims 95-132, wherein the first reporter gene encodes LacZα or functional portion thereof.

134. The recombinant bacmid of any one of claims 95-133, wherein the recombinant bacmid further comprises at least a second and a third reporter gene.

135. The recombinant bacmid of claim 134, wherein the second and the third reporter gene each encode a fluorescent protein.

136. The recombinant bacmid of claim 135, wherein the fluorescent protein is a green fluorescent protein or a red fluorescent protein.

137. The recombinant bacmid of any one of claims 95-136, wherein the first and the second preferential target site each comprise a LoxP site or a variant thereof.

138. The recombinant bacmid of any one of claims 95-137, wherein the site-specific recombination event is mediated by a Cre recombinase.

139. A recombinant bacmid comprising:
a sequence encoding a Rep, wherein the inserted Rep sequence disrupts the reading frame of the LacZα gene or functional portion thereof; and
a heterologous sequence comprising, from 5' to 3':
a wild-type or truncated 5' inverted terminal repeat derived from a first genome of a member of the viral family Parvoviridae;
a sequence encoding a protein;
one or more expression control sequences operably linked to the sequence encoding a protein; and
a wild-type or truncated 3' inverted terminal repeat derived from a second genome of a member of the viral family Parvoviridae.

140. The recombinant bacmid of claim 139, wherein the first and the second genome of the viral family Parvoviridae are the same.

141. The recombinant bacmid of claim 140, wherein the first and the second genome of the viral family Parvoviridae are different.

142. The recombinant bacmid of any one of claims 139-141, wherein the Rep is derived from the first or the second genome of the member of the viral family Parvoviridae.

143. A recombinant bacmid comprising:
a sequence encoding a B19 Rep, wherein the inserted B19 Rep sequence disrupts the reading frame of the LacZα gene or functional portion thereof; and
a heterologous sequence comprising from 5' to 3':
a wild-type or truncated 5' inverted terminal repeat derived from B19;
a sequence encoding a protein;
one or more expression control sequences operably linked to the sequence encoding a protein; and
a wild-type or truncated 3' inverted terminal repeat derived from B19.

144. A recombinant bacmid comprising:
a sequence encoding a GPV Rep, wherein the inserted GPV Rep sequence disrupts the reading frame of the LacZα gene or functional portion thereof; and
a heterologous sequence comprising from 5' to 3':
a wild-type or truncated 5' inverted terminal repeat derived from GPV;
a sequence encoding a protein;
one or more expression control sequences operably linked to the sequence encoding a protein; and
a wild-type or truncated 3' inverted terminal repeat derived from GPV.

145. A recombinant bacmid comprising:
a sequence encoding an AAV2 Rep, wherein the inserted AAV2 Rep sequence disrupts the reading frame of the LacZα gene or functional portion thereof; and
a heterologous sequence, wherein the heterologous sequence comprises from 5' to 3':
a wild-type or truncated 5' inverted terminal repeat derived from AAV2;
a sequence encoding a protein;
one or more expression control sequences operably linked to the sequence encoding a protein; and
a wild-type or truncated 3' inverted terminal repeat derived from AAV2.

146. A recombinant bacmid comprising:

a sequence encoding an HBoV1 Rep, wherein the inserted HBoV1 Rep sequence disrupts the reading frame of the LacZα gene or functional portion thereof; and
a heterologous sequence comprising from 5' to 3':
a wild-type or truncated 5' inverted terminal repeat derived from HBoV1;
a sequence encoding a protein;
one or more expression control sequences operably linked to the sequence encoding a protein; and
a wild-type or truncated 3' inverted terminal repeat derived from HBoV1.

147. The recombinant bacmid of any one of claims 139-146, wherein the protein is a therapeutic protein.

148. The recombinant bacmid of claim 147, wherein the therapeutic protein is a clotting factor.

149. The recombinant bacmid of claim 148, wherein the clotting factor is Factor VIII (FVIII).

150. The recombinant bacmid of claim 148 or 149, wherein the clotting factor is FVIII-XTEN.

151. A recombinant bacmid comprising:
a sequence encoding a Rep protein, wherein the Rep disrupts the reading frame of a reporter gene or functional portion thereof; and
a heterologous sequence comprising the nucleotide sequence of SEQ ID NO: 20.

152. The recombinant bacmid of claim 147, wherein the heterologous sequence comprises the nucleotide sequence of SEQ ID NO: 19.

153. A recombinant bacmid comprising:
a sequence encoding a Rep protein, wherein the Rep disrupts the reading frame of a reporter gene or functional portion thereof; and
a heterologous sequence comprising the nucleotide sequence of SEQ ID NO: 29.

154. A host cell comprising the bacmid of any one of claims 1-18.

155. A host cell comprising the recombinant bacmid of any one of claims 19-44.

156. A host cell comprising the vector of any one of claims 45-55.

157. A host cell comprising the vector of any one of claims 56-94.

158. A host cell comprising the recombinant bacmid of any one of claims 95-153.

159. A method of generating the recombinant bacmid of any one of claims 19-44, comprising:

introducing into a bacterial cell:

the bacmid of any one of claims 1-18; and

a donor nucleic acid molecule comprising a bacterial replicon operably linked to a transposon comprising the heterologous sequence of any one of claims 19-44;

incubating the bacterial cell under conditions for transposition to occur; and

isolating the recombinant bacmid from the bacterial cell.

160. A method of generating the recombinant bacmid of any one of claims 95-153, comprising:

introducing into a bacterial cell:

the recombinant bacmid of any one of claims 19-44;

the vector of any one of claims 56-94; and

a Cre recombinase;

incubating the bacterial cell under conditions for site-specific recombination to occur; and

isolating the recombinant bacmid from the bacterial cell.

161. A method of generating a recombinant baculovirus comprising transfecting the recombinant bacmid of any one of claims 95-153 into an insect cell under appropriate conditions.

162. A method of generating a closed ended DNA (ceDNA) molecule comprising:

infecting an insect cell with a recombinant baculovirus comprising the bacmid of any one of claims 1-44 and 95-153.

163. A method of generating a closed ended DNA (ceDNA) molecule comprising:

transfecting the recombinant bacmid of any one of claims 139-153 into an insect cell under appropriate conditions to generate a recombinant baculovirus; and

infecting a second insect cell with the recombinant baculovirus under appropriate conditions to generate a ceDNA molecule.

164. The method of any one of claims 161-163 wherein the recombinant baculovirus comprises a single bacmid.

165. A method of generating a closed ended DNA (ceDNA) molecule comprising a wild-type or truncated B19 inverted terminal repeats, comprising:
transfecting the recombinant bacmid of claim 139 into an insect cell under appropriate conditions to generate a recombinant baculovirus; and
infecting a second insect cell with the recombinant baculovirus under appropriate conditions to generate a ceDNA molecule.

166. A method of generating a closed ended DNA (ceDNA) molecule comprising a wild-type or truncated GPV inverted terminal repeats, comprising:
transfecting the recombinant bacmid of claim 139 into an insect cell under appropriate conditions to generate a recombinant baculovirus; and
infecting a second insect cell with the recombinant baculovirus under appropriate conditions to generate a ceDNA molecule.

167. A method of generating a closed ended DNA (ceDNA) molecule comprising a wild-type or truncated AAV2 inverted terminal repeats, comprising:
transfecting the recombinant bacmid of claim 139 into an insect cell under appropriate conditions to generate a recombinant baculovirus; and
infecting a second insect cell with the recombinant baculovirus under appropriate conditions to generate a ceDNA molecule.

168. A method of generating a closed ended DNA (ceDNA) molecule comprising a wild-type HBoV1 inverted terminal repeats, comprising:
transfecting the recombinant bacmid of claim 139 into an insect cell under appropriate conditions to generate a recombinant baculovirus; and
infecting a second insect cell with the recombinant baculovirus under appropriate conditions to generate a ceDNA molecule.

169. A plasmid comprising a nucleic acid sequence comprising from 5' to 3':
a wild-type or truncated 5' inverted terminal repeat derived from a genome of a first member of the viral family Parvoviridae;
a sequence encoding a protein;
one or more expression control sequences operably linked to the sequence encoding a protein; and
a wild-type or truncated 3' inverted terminal repeat derived from a genome of a second member of the viral family Parvoviridae.

170. The plasmid of claim 169, wherein the one or more expression control sequences comprise a tissue-specific promoter, a polyadenylation signal, and/or a post-transcriptional regulatory element.

171. The plasmid of claim 170, wherein the tissue-specific promoter is a tristetraprolin (TTP) or a murine transthyretin (mTTR) promoter.

172. The plasmid of claim 169 or 170, wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal.

173. The plasmid of any one of claims 169-172, wherein the post-transcriptional regulatory element is a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE).

174. The plasmid of any one of claims 169-173, wherein the protein is a therapeutic protein.

175. The plasmid of claim 174, wherein the therapeutic protein is a clotting factor.

176. The plasmid of claim 175, wherein the clotting factor is Factor VIII (FVIII).

177. The plasmid of claim 175 or 176, wherein the clotting factor is FVIII-XTEN.

178. The plasmid of any one of claims 175-177, wherein the clotting factor comprises the nucleotide sequence of of SEQ ID NO: 20.

179. The plasmid of any one of claims 169-178, wherein the first and/or the second members are selected from the group consisting of B19, GPV, HBoV1, and AAV2.

180. The plasmid of claim 179, wherein the first and/or the second members are the same.

181. The plasmid of claim 179, wherein the first and/or the second members are different.

182. A stable cell line comprising the nucleic acid sequence of any one of claims 169-181, wherein the nucleic acid sequence is stably integrated in the genome of the stable cell line.

183. The stable cell line of claim 182, wherein the stable cell line is a stable insect cell line.

184. The stable cell line of claim 183, wherein the stable insect cell line is Sf9.

185. A method of generating a closed ended DNA (ceDNA) molecule comprising wild-type or truncated inverted terminal repeats, comprising introducing into the stable cell line of any one of claims 182-184 a baculovirus encoding a Rep protein.

186. The method of claim 185, wherein the stable cell line is an insect cell line.

187. The method of claim 186, wherein the insect cell line is Sf9.

188. A set of recombinant bacmids comprising a first bacmid and a second bacmid,
wherein the first bacmid comprises a sequence encoding a Rep inserted into a mini-attTn7 site, wherein the inserted Rep disrupts the reading frame of a reporter gene or functional portion thereof; and
wherein the second bacmid comprises a heterologous sequence, wherein the heterologous sequence comprises from 5' to 3':
a wild-type or truncated 5' inverted terminal repeat (ITR) derived from a first genome of a member of the viral family Parvoviridae;
a sequence encoding a protein;
one or more expression control sequences operably linked to the sequence encoding a protein; and

a wild-type or truncated 3' inverted terminal repeat (ITR) derived from a second genome of a member of the viral family Parvoviridae.

189. The set of recombinant bacmids of claim 188, wherein the 5' ITR and the 3' ITR are derived from a parvovirus selected from the group consisting of B19, GPV, HBoV1, and AAV2.

190. The set of recombinant bacmids of claim 188 or 189, wherein the heterologous sequence comprises the nucleotide sequence of SEQ ID NO: 20.

191. The set of recombinant bacmids of claim 188 or 189, wherein the heterologous sequence comprises the nucleotide sequence of SEQ ID NO: 19.

192. The set of recombinant bacmids of claim 188 or 189, wherein the heterologous sequence comprises the nucleotide sequence of SEQ ID NO: 29.

193. A method of generating a closed ended DNA (ceDNA) molecule comprising:
infecting an insect cell with a recombinant baculovirus comprising the set of recombinant bacmids of any one of claims 188-192.

194. A method of generating a closed ended DNA (ceDNA) molecule comprising:
transfecting the set of recombinant bacmids of any one of claims 188-192 into an insect cell under appropriate conditions to generate a recombinant baculovirus; and
infecting a second insect cell with the recombinant baculovirus under appropriate conditions to generate a ceDNA molecule.

195. A recombinant bacmid comprising:
a bacterial replicon;
a first antibiotic resistance gene;
a sequence encoding a HBoV1 Rep, wherein the inserted HBoV1 Rep sequence disrupts the reading frame of the LacZα gene or functional portion thereof;
a gene encoding a fluorescent protein operably linked to a baculovirus-inducible promoter; and
a LoxP site or a variant thereof.