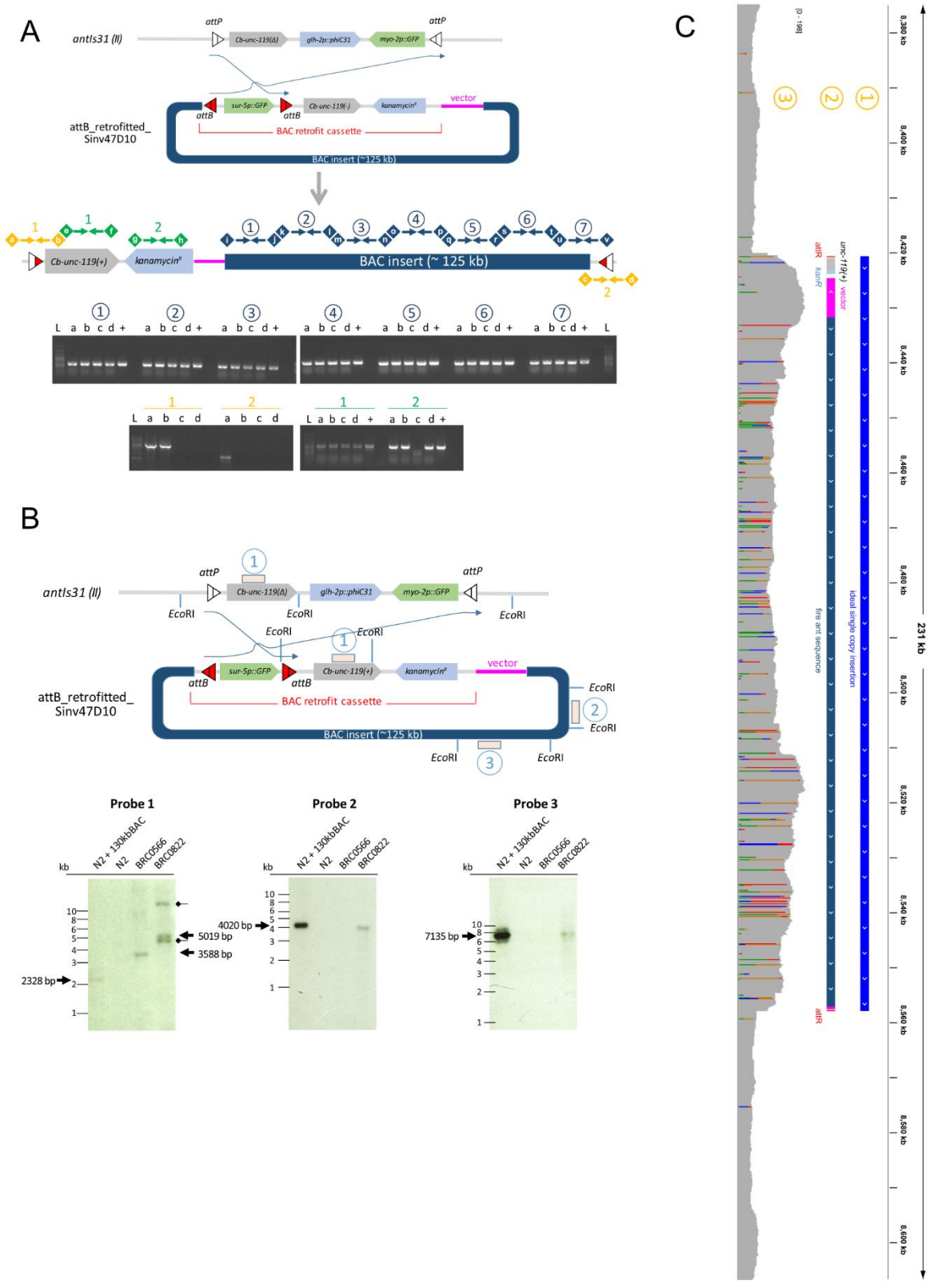
Supplementary Figure S7



Supplementary Figure S7. Large BAC integration attempt. (A) Top, Schematic for RMCE of attB_retrofitted_Sinv47D10 BAC (BAC 47D10 retrofitted with an attB cassette) into the *antIs31* docking site. Proper insertion would insert ~137.2 kb, comprising ~125.4 kb of fire ant sequence and ~11.8 kb of retrofitted vector. Bottom, Representative PCR validation assays. BAC PCR assays were spaced 10-15 kb apart and each amplified ~500-600 bp. These 4 BAC insertion lines were positive for all 7 PCR assays within the BAC, however all lines were also GFP positive. Only BAC insertion line 'a' has the proper recombination junctions (orange PCR #1). These data suggest that the BAC insertions were complex and possibly multicopy. The primer sequences are listed in **Supplementary Table S3**. (B) Southern blot examining strain BRC0882 (*antSi33*), which was positive for all 7 BAC PCR assays and did not express GFP. Top, Schematic shows relevant *Eco*RI restriction sites and probe sites (not to scale). Bottom, genomic DNA from N2, BRC0566 (*antIs31*), and BRC0882 (*antSi33*) as well as attB_retrofitted_Sinv47D10 BAC DNA was digested with *Eco*RI, and subjected to Southern blot assays using the indicated probes. Left, probe #1 hybridized to a 2,328 bp fragment on the BAC and a 3,588 bp fragment on BRC0566; the probe target was not present in N2, as expected. In the integrant strain BRC0882, the probe hybridized to a presumably correct 5,019 bp fragment as well as 2 additional fragments. This suggests that the integrant is in low copy with some rearrangements among the copies. Middle and right, probes #2 and #3 hybridized to single fragments of predicted sizes 4,020 bp and 7,135 bp, respectively, on both the BAC and the integrant. Observing a single band is consistent with a single-copy insertion or a low copy number insertion with no rearrangement in this region. Normal arrows point to predicted fragments with sizes indicated in parentheses; diamond headed arrows indicate unexpected fragments. (C) IGV plot of Oxford Nanopore (ONT) sequence reads shows that the *antSi33* region has greater read depth than flanking regions. There appear to be ~2-4 copies of the BAC depending on the subregion. ① Blue bar shows the extent of the ideal single copy insertion. ② Sub-sections of the ideal insertion as labeled. ③ Gray is coverage depth across the insert and the flanking region. Colored vertical bars are potential mutational differences between the predicted BRC0882 genome and the sequence reads. The sequence for the attB_retrofitted_Sinv47D10 BAC was assembled and polished from ONT reads only, so some potential mutations could be assembly errors.