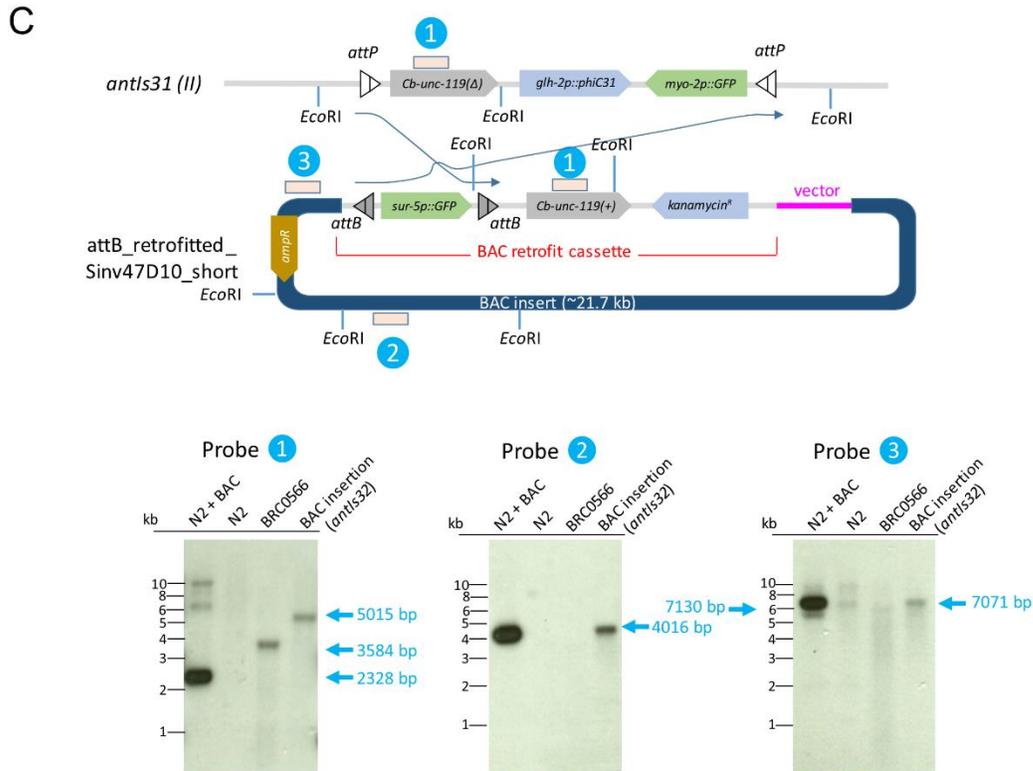


Supplementary Figure S6 (cont)



Supplementary Figure S6. Validation of 33.4 kb BAC integration. (A) Top, Schematic for RMCE of *attB_retrofitted_Sinv47D10_short* BAC (BAC 47D10 retrofitted with an *attB* cassette and then shortened) into the *antIs31* docking site. Proper insertion would insert ~33.4 kb, comprising ~20.7 kb of fire ant sequence, ~0.9 kb of *ampR*, and ~11.8 kb of retrofitted vector. Relative locations for the PCR assays are shown. PCR assays on the BAC were spaced 3-4 kb apart and each amplified ~500 bp. Schematic not to scale. (B) Representative PCR validation assays. Top, results for four candidate BAC insertion lines that were all GFP negative (labeled 'a' to 'd') for the two junctions, *unc-119*, and *kanR*. Bottom, results for lines 'b', 'c', and 'd' for the 5 BAC assays. BAC assays for line 'a' were also all positive but not shown since its junction #1 PCR results were incorrect. For the junction assays, the light (junction 1) and dark (junction 2) orange arrows indicate the correct PCR product. The diamond headed arrow indicates an incorrect PCR product for 'a'. BAC insertion 'b' corresponds to *antIs32*, which is shown in Fig. 3 in the main text. The primer sequences are listed in **Supplementary Table S3**. (C) Southern blot examining *antIs32*. Top, Schematic shows relevant *EcoRI* restriction sites and probe sites (blue numbered circles). Bottom, Genomic DNA from N2, BRC0566 (*antIs31*), and BRC0906 (*antIs32*) as well as *attB_retrofitted_Sinv47D10_short* BAC DNA was digested with *EcoRI*, and subjected to Southern blot assays using the indicated probes. Left, probe #1 hybridized to a ~2 kb fragment on the BAC, a ~3.5 kb fragment on BRC0566, and a ~5 kb fragment in the integrant strain BRC0906; the probe target was not present in N2, as expected. Middle (same as Fig. 3B), probe #2 hybridized to an ~4 kb fragment on the BAC and the integrant. Right, probe #3 hybridized to an ~7 kb fragment on the BAC and the integrant. Expected fragment sizes are indicated next to the arrows. Observing a single band with all three probes in the integrant is consistent with a single-copy insertion or a low copy number insertion with no rearrangement in the probed region.