SUPPLEMENTAL MATERIAL

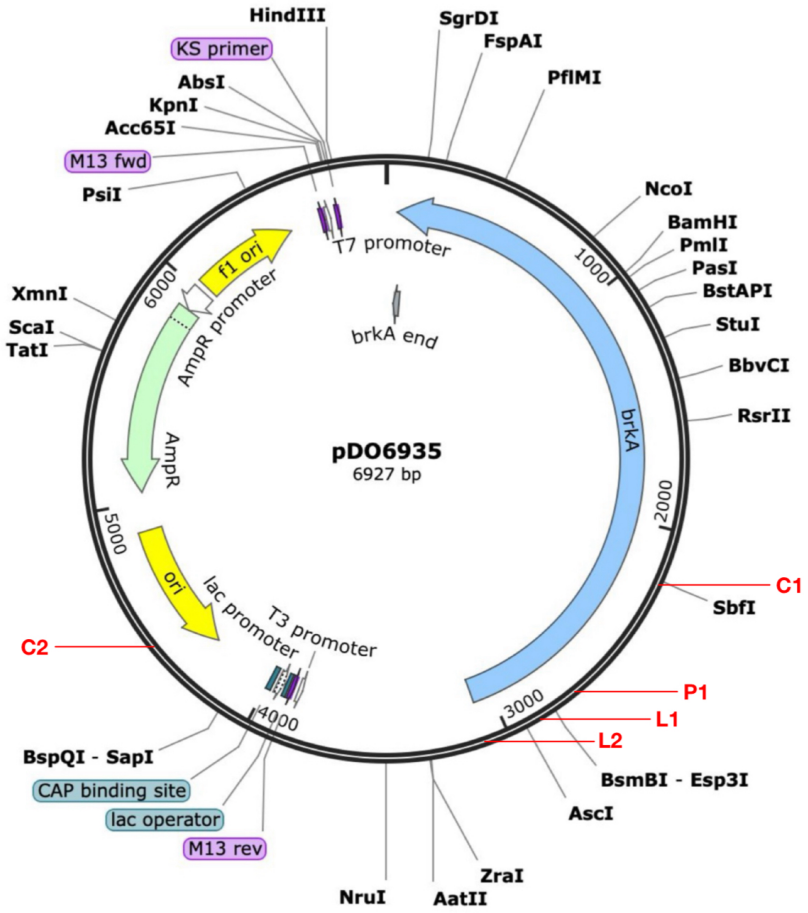

FIG. S1 A plasmid map of pDO6935 with specific primer target sites labelled. PCR amplification targeting a 2312 bp region encompassing the cDNA sequence was performed using forward primer C1 (binding at nucleotide positions 2197-2218) and reverse primer C2 (binding at nucleotide positions 4486-4508) of pDO6935. Primer P1 binds to nucleotide positions 2773-2790, downstream of the *brkA* translation start site, ensuring the inclusion of the *brkA* gene's beginning and the upstream sequence, potentially containing the transcription start site (TSS). Custom forward and reverse primers, L1 and L2, were used to validate the presence of the ligated cDNA fragment containing primer P1 and TSS sequences. The nucleotide sequences of the primers are available in Table 1.

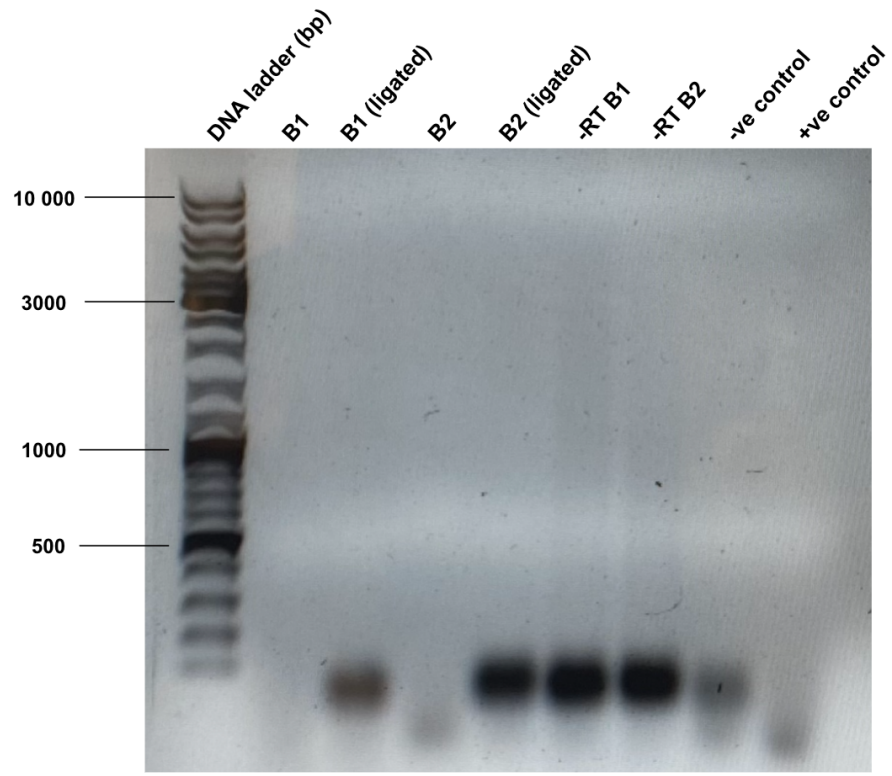

FIG. S2 PCR check for cDNA ligation with reduced amount of template shows lack of amplification in all lanes. PCR was performed on unligated (lanes 2 and 4) and ligated (lanes 3 and 5) cDNA samples, alongside negative reverse transcriptase (-RT) controls (lanes 6 and 7), a negative no-template control (lane 8), and a positive control using pDO6935 plasmid (lane 9). Volumes of cDNA template samples for PCR were reduced by half from the previous attempt (Figure 3). Primers L1 (forward) and L2 (reverse) were used to amplify an 800 bp region of only the ligated cDNA. PCR products were run on a 1% agarose gel. All lanes show the lack of amplification, including the positive control lane.

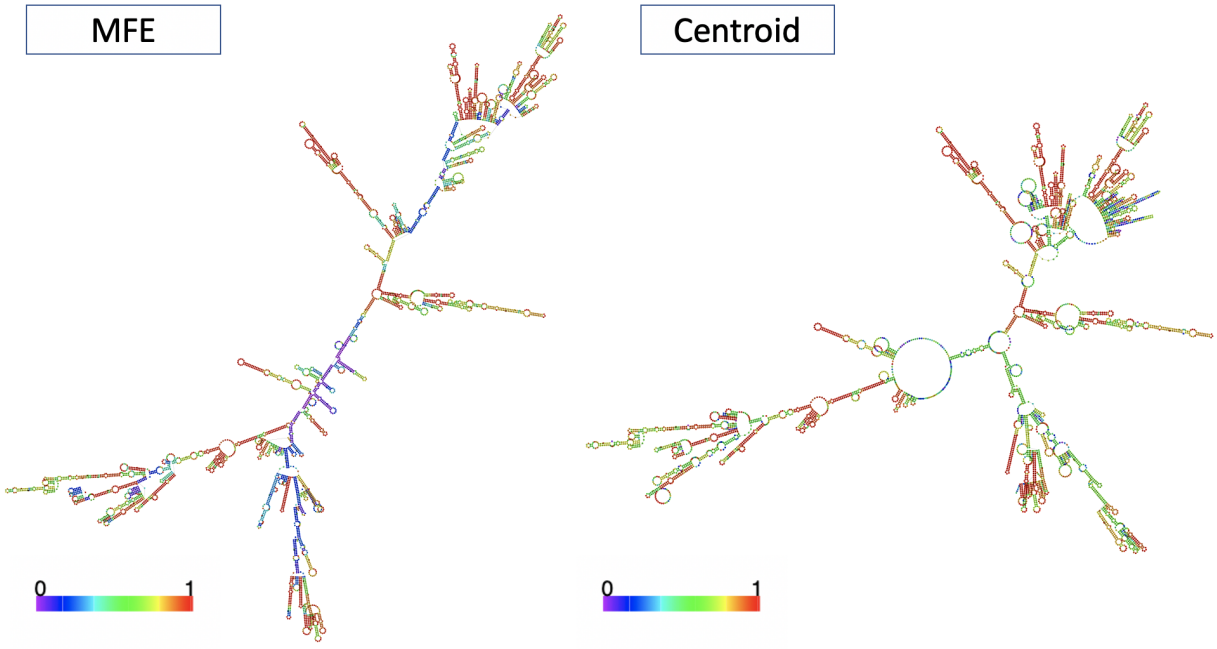

FIG. S3 RNAfold indicates secondary structure formation in *brkA* cDNA. Sidhu *et al.*'s longest sequence of *brkA* cDNA was inputted in RNAfold WebServer to detect secondary structure formation. MFE (minimum free energy) secondary structure refers to the cDNA structure that has the lowest free energy among all possible secondary structures. Centroid secondary structure represents the collective average of all predicted *brkA* secondary structures, consolidating them into a single structural representation. This figure indicates regions of potential secondary structure formation by determining base-pairing probability of the input *brkA* cDNA sequence. Blue regions have a probability of 0 for base pairing prediction, and red has a probability of 1 for base pairing prediction.