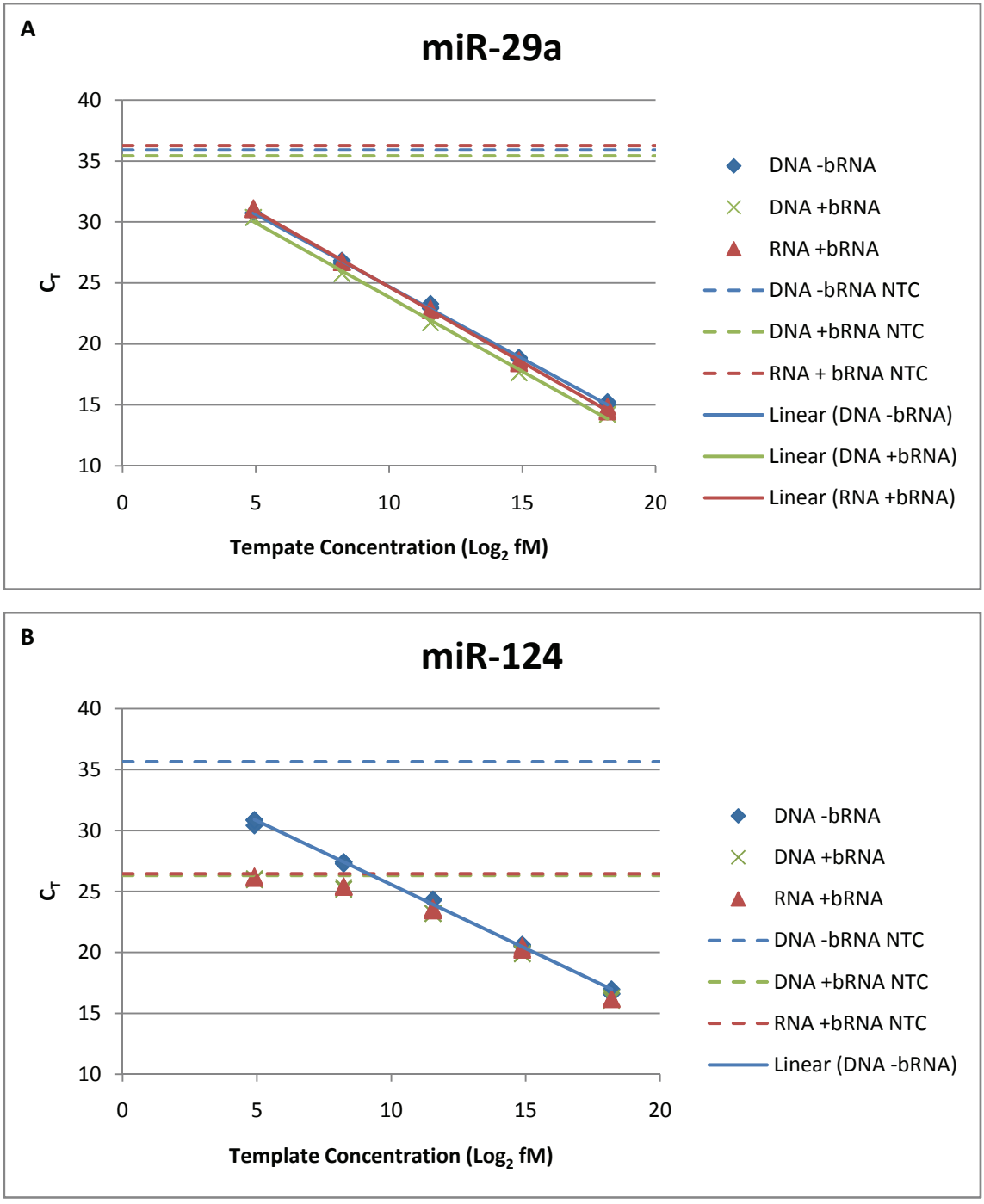

Supplementary Figure: Comparison of RNA and DNA templates for qRT-PCR miRNA Assay.

Five point standard curves were prepared from either DNA or RNA oligonucleotides corresponding to the sequence of mature miR-29a (A) or miR-124 (B), ranging from 300 pM to 30 fM. To test the effect of 'background' RNA, standard curves were either prepared in nuclease free water (-bRNA) or in 10 ng/µl total *E. coli* RNA (+bRNA). Samples were assayed as described in Materials and Methods, and plotted here as C_T value against $\log_2$ of the template concentration in fM. The mean C_T value of the No Template Control (NTC) is shown as a dashed line for each standard curve. Linear regression slopes are shown where appropriate.

The test for miR-29a showed very little difference between DNA and RNA templates, either with or without background RNA present. The results for miR-124 were complicated by the detection of a signal in the no template control (NTC) of samples containing *E. coli* RNA. We presume that *E. coli* contain a species of RNA sufficiently similar and abundant to be detected by this assay. The DNA and RNA curves with background RNA responded virtually identically, and the three highest concentrations responded very similarly to the DNA template without background RNA. Whilst it is possible that across longer templates, DNA and RNA are transcribed with different efficiencies, across the very short templates used for the miRNA assay, and under our experimental conditions there is no clear difference between the two template types.

The inclusion of background RNA did not have a substantial effect on the response to the standard curve, except considerably increasing the background for the miR-124 assay. This is most likely due to the relatively low concentration of total RNA used in this assay (3.33 ng/µl final conc. total RNA).