

6.5. Studying the interactions of Gp39 and Gp40

As the *in vivo* degradation assays showed no degradation for Gp39 and was somewhat inconclusive for Gp40, to try and gain some insight into how they appeared in the pull down assay with the ClpC DWB trap mutant we decided to investigate whether they were able to interact with Gp53 and/or ClpC using bacterial two-hybrid and bacterial three-hybrid assays.

6.5.1. Bacterial two-hybrid assay of Gp39 and Gp40 with Gp53

Firstly, Gp39 and Gp40 were cloned into the bacterial two-hybrid vector pUT18 to create fusions with the T18 domain of the adenylate cyclase. Once the vectors were created bacterial two-hybrid assays were set up to test whether these proteins interact with Gp53. Interestingly, as can be seen in Figure 6.8, Gp40 significantly interacts with Gp53 but not quite to the extent that is seen with ClpC, perhaps indicating more of a weak interaction. Conversely, Gp39 showed no significant interaction with Gp53 (Figure 6.8).