



**Figure S5** Quantitative retesting of transformability in recombinant clones. Values Kan<sup>R</sup>/CFU transformation frequencies for MIV starvation cultures and represent the mean of triplicate experiments and error bars show  $\pm$  standard deviation. Letters indicate significant differences from the Rd recipient strain by paired t-test. **(A)** Retest of the seven recombinants that had shown a reduced transformation frequency in the primary ‘transformation-during-growth’ assay (purple dots in **Figure 10A**). **(B)** Retest of the six recombinants for which donor segments spanned all or part of a known competence genes. Genes spanned by donor segments are as follows: RR4108 spanned *comM*; RR4055 spanned *sxy* and *rec1*; RR4017 spanned part of *pilA*; RR4085 spanned part of *pilB* and all of *pilCD*; RR4071 spanned *comNOPQ* and *radC*; and RR4078 spanned *ligA*. Known competence-regulated genes not spanned by donor segments were: *comABCDEF*, *comE1*, *pilF2*, *rec2*, and the unknowns *HI0659*, *HI0660*, *HI1631*, and *HI0365*.