Supplementary Figure 2. Integration of the pCG6-attB plasmid into the hrp3 promoter by homologous recombination. (a) Schematic diagram of pCG6-attB plasmid integration into the hrp3 locus on chromosome 13. Several clones obtained from limiting dilution of the transfected line Dd2<sup>attB</sup> were identified as hrp3 integrants during routine PCR screening. Primers p32 (a forward primer to the PBS backbone) and p33 (a reverse primer to the hrp3 protein coding region) amplified a 2.0 kb product (data not shown). The recombinant locus introduced the human DHFR selectable marker, a 3' UTR from hrp2, and the attB site in the upstream locus, and reconstituted the wild type hrp3 in the downstream locus. (b) Southern mapping of genomic DNA from Dd2 hrp3 integrants confirmed single copy insertion of the pCG6-attB plasmid into the hrp3 locus. Genomic DNA was digested with BciI enzyme to liberate the entire hrp3 locus. Hybridization with a hrp3 5' UTR probe (position shown, isolated from plasmid pILH10) indicated complete replacement of the wild type 7.5 kb locus with the 14 kb recombinant locus in Dd2<sup>attB/hrp3</sup>. Lane 1, Dd2; 2, Dd2<sup>attB/hrp3</sup>; 3, pCG6-attB plasmid. Additional hrp3 integrants (3D7<sup>attB/hrp3</sup>) were identified in the 3D7 background (Table 1; data not shown).