Bacillus subtilis 168 was developed as a genome vector to manipulate large DNA fragments. The system is based on the inherent natural transformation (TF) activity. However, DNA size transferred by TF is limited up to approximately 100 kb. A conjugal transfer system capable of transferring DNA fragments considerably larger than those transferred by TF was developed. A well-defined *oriT*^{110} sequence and a cognate relaxase gene from the pUB110 plasmid were inserted into the *xkd*E gene of the *B. subtilis* genome. Transfer of antibiotic resistance markers distant from the *oriT*^{110} locus to the recipient *B. subtilis* occurred only in the presence of pLS20, a helper plasmid that provides a type IV secretion system. Marker transmission was consistent with the orientation of *oriT*^{110} and required a *recA*-proficient recipient. The first conjugal transfer system of genomic DNA should provide a valuable alternative genetic tool for editing the *B. subtilis* genome.

The first conjugal system to transfer the *Bacillus subtilis* genome like an Hfr mechanism. A conjugal plasmid pLS20 makes the genome-integrated oriT active.