



PCR product (pCS1-f 1/pCS2-r 1) XXXGAAAAACGTCCTAGGGTAGCTAAGCTTAAAGGATCCAAGGCCGCCACCATG*c* *ggcgcgca*GGTGGCGGAGGTGGCGTGAGCAAGGGCGAGGAGCTGTTCCXXX
 XXXCTTTTTGCAGGATCCCATCGATTTCGAATTCCATAGGTTCCGGCGGTGGTAC*g* *ccggcgct*CCACCGCTCCACCGCACTCGTTCCCGCTCCTCGACAAGXXX

PCR product (pCS1-f 2/pCS2-r 2) XXXGAAAAACGTCCTAGGGTAGCTAAGCTTAAAGGATCCAAGGCCGCCACCATG*c**ta* *gca*GGTGGCGGAGGTGGCGTGAGCAAGGGCGAGGAGCTGTTCCXXX
 XXXCTTTTTGCAGGATCCCATCGATTTCGAATTCCATAGGTTCCGGCGGTGGTAC*gat* *cg*tCCACCGCTCCACCGCACTCGTTCCCGCTCCTCGACAAGXXX

denaturation and annealing

Bfal /NotI digested sticky end

XXXGAAAAACGTCCTAGGGTAGCTAAGCTTAAAGGATCCAAGGCCGCCACCATG*c* *ggcgcgca*GGTGGCGGAGGTGGCGTGAGCAAGGGCGAGGAGCTGTTCCXXX
 XXXCTTTTTGCAGGATCCCATCGATTTCGAATTCCATAGGTTCCGGCGGTGGTAC*gat* *cg*tCCACCGCTCCACCGCACTCGTTCCCGCTCCTCGACAAGXXX

ligation

