

70.II-ISceI Construction

"70.IIRS_GHMFw1":

AATAGGAGAGTGTGGTGTGAGTGTGTGCTTACCAATATTATAATAATGATAGTAACGACCAAT
AGGGATAACAGGGTAATACCGGTATAACTTCGTATAGCATAACATTATACGAAGTTATCTCG

"70.IIRS_GHMRv1":

CAAATCCATTATACTCACGATTACTCTCATTGCACACATACCATTGTCTTAACTGCATTTAT
TTATGGTTTGTATTTCGTACATAACTTCGTATAATGTATGCTATACGAAGTTATCCTCTAATCC
TTTCACTCTTCCG

70.I-ISceI Construction

"70.IRS_Fw1":

ATATGCGGCCGCATGACAGCAGTTTTAGACACTAAAGGCGGAGCCCAAATAGAGGCACTT
ACAACCTTTAGAAAAAATTAATTAGAGTAGGGATAACAGGGTAATACCGGTATAACTTCGT
ATAGCATAACATTATACGAAGTTATCTCG

"70.IRS_Rv1":

ATATGCGGCCGCATGCAGCTGCGCATATACAATATCACTTTCTATATTTGTCTTTCTGCGT
TTCCTTTAATATATTTCTAAGTGCGCTCCAACCTTACATAACTTCGTATAATGTATGCTATAC
GAAGTTATCCTCTAATCCTTTCACTCTTCCG

Δ70-ISceI Construction: Flanking regions were sewn to PURO cassette by long-flanking homology PCR. Oligos for amplification of the 5' and 3' flanking homology regions are shown. PCR product resulted in ~1.5Kb of homology to BES1 on each side of cassette.

5'-Flanking homology

"NotI ESAG1 UQ Fw":

ATATGCGGCCGCATGGAAGAGCAAACCTGATAGGTTGGAAAAGC

"-70 P2 NEW":

CCCTCGAGATAACTTCGTATAATGTATGCTATACGAAGTTATACCGGTATTACCCTGTTAT
CCCTACTTTTAACCTTGTGCCCCGACCTTTACAGTC

3'-Flanking homology

"-70 P2 #3 (KpnI)":

CCCTCGAGATAACTTCGTATAATGTATGCTATACGAAGTTATTATTACCCTGTTATCCCTAG
GTACCCTTTTAACCTTGTGCCCCGACCTTTACAGTC

"VSG221 5'NotI Rv1":

ATATGCGGCCGCATGGAAGAACTTGGGCTAGGACCAAGACG

Monomer Oligo

6-77-Fw

ATAGGTACCATAATAATAATAATAATAATAATAATAGGAGAGTGTGGTGTGAGTGTGTGTATAT
ACGAATATTATAATAAGAGCAGTAATAATAATAATAATAGGTACCATA

Dimer Oligo

2x-6-77-Fw

ATAGGTACCATAATAATAATAATAATAATAATAATAATAGGAGAGTGTGGTGTGAGTGTGTGTATAT
ACGAATATTATAATAAGAGCAGTAATAATAATAATAATAATAATAATAATAATAATAATAATAGGAGA
GTGGTGTGAGTGTGTGTATATACGAATATTATAATAAGAGCAGTAATAATAATAATAATAGGTAC
CATA

Mut_Dimer Oligo

2x-6-77mut_Fw

ATAGGTACCATAATAATAATAATAATAATAATAATAATAAAAAAAAAATTATAAATATATATATATAT
AAATATTATAATAAAAAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATA
TATAAATATATATATATAAATATTATAATAAAAAATAATAATAATAATAATAATAATAGGTACCATA