

SUPPLEMENTARY MATERIAL FOR:

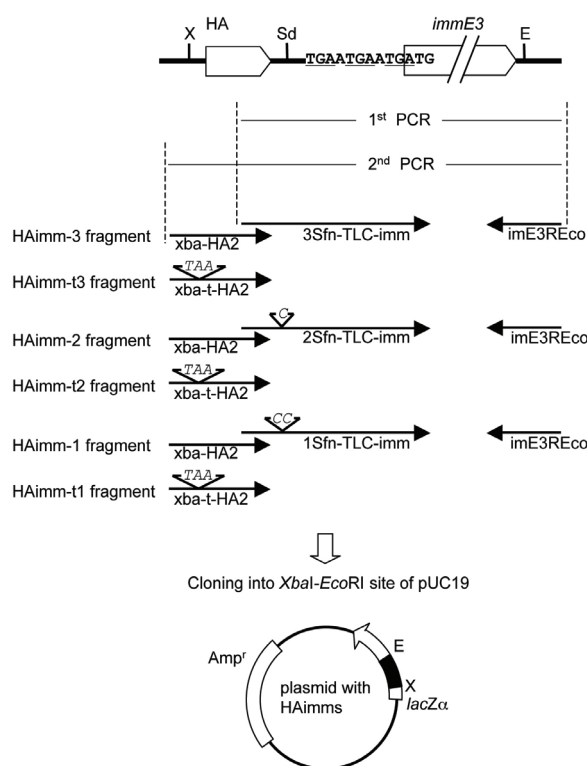
A novel vector for positive selection of inserts harboring an open reading frame by translational coupling

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BioTechniques 43:751-754 (December 2007)

doi 10.2144/000112629



Supplementary Figure S1. Construction of six plasmids with HAimms containing the influenza virus hemagglutinin (HA) epitope sequence. A fragment containing HA epitope sequence, the novel sequence, and *immE3* was prepared from pSH350 by two PCR amplifications, and cloned into the *XbaI-EcoRI* site of pUC19. Primers are indicated by arrows. Forward primers for 1st PCR:

1Sfn-TLC-imm 5'-CGTCCCAGACTACGCTCCGGCCAAAGCGGCCTGAATGAATGATGGGACTT-3';

2Sfn-TLC-imm 5'-CGTCCCAGACTACGCTCCGGCCAAAGCGGCCTGAATGAATGATGGGACTT-3';

3Sfn-TLC-imm 5'-CGTCCCAGACTACGCTGGCCAAAGCGGCCTGAATGAATGATGGGACTT-3';

forward primer for 2nd PCR:

xba-HA2

5'-ACTCTAGAGATGGGTTACCCATACGACGTCCCAGACTACGCT-3';

xba-t-HA2

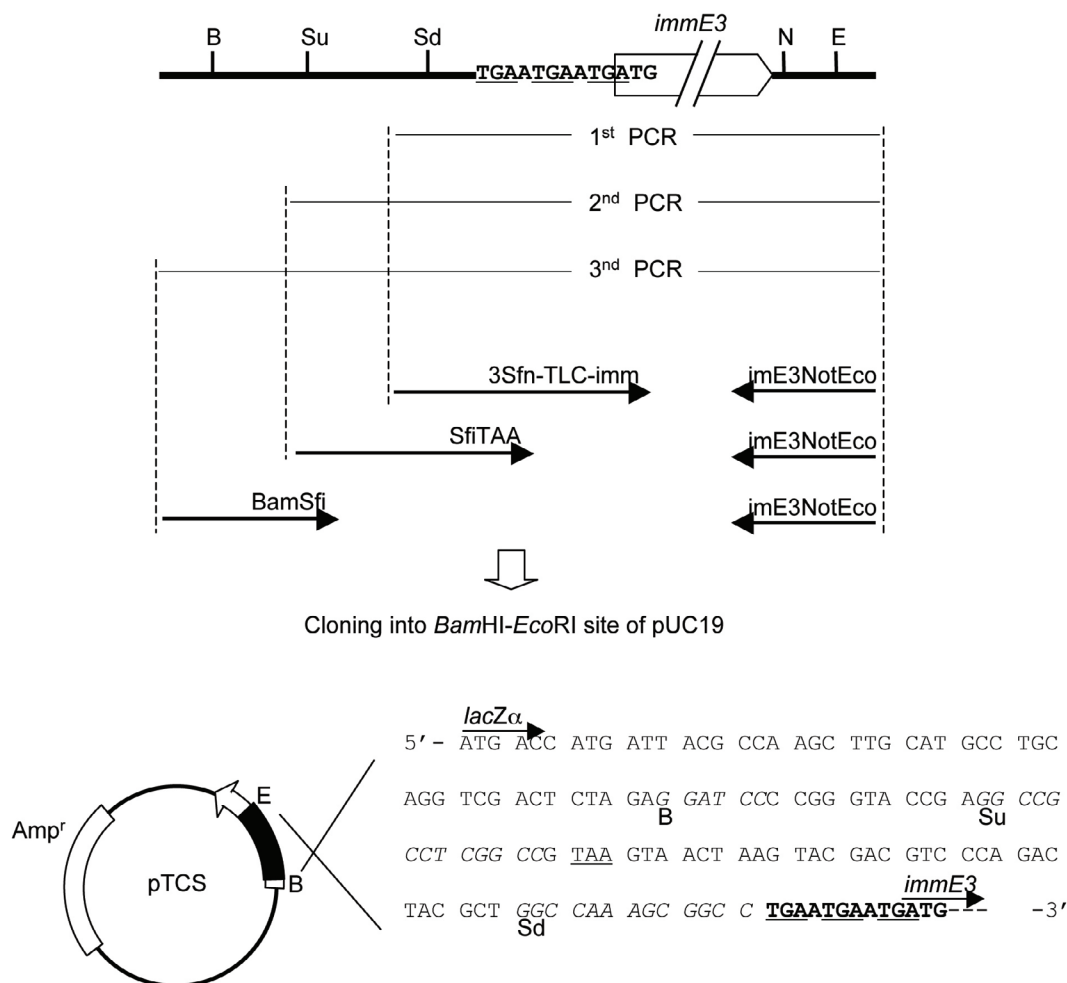
5'-ACTCTAGAGTAAATGGGTTACCCATACGACGTCCCAGACTACGCT-3';

reverse primer:

imE3REco 5'-CGATGAATTCTCACCAATCACCATCACGATAATC-3'.

CC (1Sfn-TLC-imm), C (2Sfn-TLC-imm) at 3'-end of HA epitope sequence for different downstream reading frame each other, and stop codon TAA (*xba*-t-HA2) at its 5'-end are italicized in the sequence of primers. The cloned fragment is presented by a solid line. Restriction sites are indicated as follows: X, *XbaI*; Sd, *SfiI* (down); E, *EcoRI*.

Benchmarks



Supplementary Figure S2. Construction of pTCS. A fragment containing two *Sfi*I sites, the novel sequence, and *immE3* was prepared from pSH350 by three PCR amplifications, and cloned into the *Bam*HI-*Eco*RI site of pUC19. Primers are indicated by arrows. Forward primer for 1st PCR:

3Sfn-TLC-imm 5'-CGTCCCAGACTACGCTGGCCAAAGCGGCCTGAATGAATGATGGGACTT-3';

forward primer for 2nd PCR:

*Sfi*TAA

5'-CCTCGGCCGTAAGTAACTAAGTACGACGTCCCAGACTACGCT-3';

forward primer for 3rd PCR:

*Bam*Sfi

5'-CTAGAGGATCCCCGGGTACCGAGGCCGCTCGGCCGTAAGTAAC-3';

reverse primer:

imE3NotEco

5'-CCAGTGAATTCGCGGCCGCTCACCAATCACCATCACGAT-3'.

The cloned fragment is presented by a solid line. The sequence from the initiation codon of *lacZ'* to the initiation codon of *immE3* is indicated. The novel sequence, TGAATGAATGATG, is shown in bold letters. Termination codons are underlined. Restriction sites are indicated as follows: B, *Bam*HI; Su, *Sfi*I (up); Sd, *Sfi*I (down) N, *Not*I; E, *Eco*RI. Sequences of restriction sites are italicized.

Supplementary Table S1. Sequences of Some Clones Obtained from TAA-Avi1, TAA-Avi2, and TAA-Avi3 Transformants Cultured in the Presence of Colicin E3

DNA Fragment	Mutant #	Sequence	Number of Clones
TAA-Avi1		5'-GGCCGCCTCGGCCGTAA(Avidin)GGCCAAAGCGGCC-3'	
	1	5'-GGCCGCCTCGGCCG—(Avidin)CCGGCCAAAGCGGCC-3'	6
	2	5'-GGCCGCCTCGGCCGTAC(Avidin)GGCCAAAGCGGCC-3'	2
TAA-Avi2		5'-GGCCGCCTCGGCCGTAA(Avidin)CGGCCAAAGCGGCC-3'	
	3	5'-GGCCGCCTCGGCCGTAC(Avidin)CGGCCAAAGCGGCC-3'	8
TAA-Avi3		5'-GGCCGCCTCGGCCGTAA(Avidin)CCGGCCAAAGCGGCC-3'	
	4	5'-GGCCGCCTCGGCC—A(Avidin)CCGGCCAAAGCGGCC-3'	1
	5	5'-GGCCGCCTCGGCCGTAC(Avidin)CCGGCCAAAGCGGCC-3'	1
	6	5'-GGCCGCCTCGGCCGTAT(Avidin)CCGGCCAAAGCGGCC-3'	6

Sequencing of some clones obtained from the TAA-Avi1, TAA-Avi2, and TAA-Avi3 transformants cultured in the presence of colicin E3 revealed mutations near the 5'- or 3'-termini of the inserts, allowing translational read-through. Dashes (—) indicate deleted bases. Bolding and underlining indicate point mutations and insertions, respectively. *Sfi*I sites are italicized. "(Avidin)" indicates the synthetic streptavidin-encoding gene (387 bp). The mutations shown here were likely introduced during PCR amplification or during cultivation of the transformants. However, TAA-Avi1 mutant #1 likely arose because of contamination with trace amounts of the alternative constructs that were prepared in other experiments.