Supplementary Material for

Single step BP/LR combined Gateway® reactions

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Materials and Methods

Unless otherwise noted, all reagents were from Life Technologies (Carlsbad, CA). Reactions were setup using 100 ng of each of the DNA components in a total volume of 10 μl: GeneArt® String™ DNA fragment GW1-1 (linear synthesized DNA, see sequence below, attB sequences in bold and underlined), P_{TAC}-GFP (PCR-amplified fragment harboring a GFP nucleotide sequence preceded by the TAC promoter, see sequence below, attB sequences in bold and underlined), pDONR™221 (donor vector), pEXP1-DEST (destination vector) and the specified amounts of Gateway® BP Clonase® II and Gateway® LR Clonase® II as indicated in the text and figure. Reactions were performed following the recommendations stated in the corresponding manuals, with two exceptions: 1) incubations were performed for 3 h at 25°C, and 2) where noted, the indicated enzyme ratios were employed. After completion, 2μl reaction samples were transformed into One Shot® OmniMAX™ 2 T1R Chemically Competent Escherichia coli cells and plated onto LB ampicillin x-gal and LB kanamycin x-gal agar plates following the manufacturer’s protocol. M13-forward and reverse oligonucleotide sequences are 5’-GTAAAACGACGGCCAG-3’ and 5’-CAGGAAAACAGCTATGAC-3’ respectively.

Sequence of GW1-1

GGGACAAGTTTTGTACAAAAAAGCAGGTTATACCTGCAGTCCGCGGAGCGATTTTGATGTTACACCTGAGCAAGCTGTACGAGCTTTCTTGTACAAAGTGG

Sequence of P_{TAC}-GFP

GGGACAAGTTTTGTACAAAAAAGCAGGCTTACGTGCTTCTGAGGGCCGAGCGGAGCTATGAGTGTTACACCTGAGCAAGCTACGTGAGCTTTCTTGTACAAAGTGG

GTCACTATATGAAACGGCATGACTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATACTTTCAAAGATGACGGGAACTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCTTAAAACAAATCTTTCACTGCAGTCCGCGGAGCGGAGCTATGAGTGTTACACCTGAGCAAGCTACGTGAGCTTTCTTGTACAAAGTGG

GGATCATATGAAACGGCATGACTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATACTTTCAAAGATGACGGGAACTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCTTAAAACAAATCTTTCACTGCAGTCCGCGGAGCGGAGCTATGAGTGTTACACCTGAGCAAGCTACGTGAGCTTTCTTGTACAAAGTGG

TCGCCCTAAAACAAATCTTTCACTGCAGTCCGCGGAGCGGAGCTATGAGTGTTACACCTGAGCAAGCTACGTGAGCTTTCTTGTACAAAGTGG
GTATCGAGTTAAAAGGTATTGATTCTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAGTACAACA
TAACTCACACAATGTATACATCAGGCAGACAAAACAAAGATGGAATCAAAGCTAACTTCAAAATTCGC
CACAACATTGAGATGGATCCGTTCAACTAGCAGACCATTATCAACATTACATCCAATTTGCGATGCC
CTTGCTCTTTACCAGACAACCATTACCTGTGCAAGACAATCTGCCCCTTTCCAAGATCCCAACGAAAAGCG
TGACCACATGGTCTTCTTGTAGTTTGTAACTGCTGCTGGGATTACACATGGGATGGATGCTCTACAAA
TAATATATGAGGCACAAGACTACCAGCTTTCTTGTACAAAGTGGTCCCC