Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Enzymatic synthesis of sialylation substrates powered by a novel polyphosphate kinase (PPK3)

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Materials

HotStarTaq Master Mix Kit, QIAquick PCR Purification Kit, QIAprep Spin Miniprep Kit were from QIAGEN (Hilden, Germany). The linearized plasmid vector pET-34b(+) and T4 DNA Polymerase, that is suitable for ligation-independent cloning, were from Novagen (Madison, WI). *S. pomeroyi* and *H. ducreyi* genomic DNA were obtained from American Type Culture Collection (ATCC 700808D). Primers were from Invitrogen (Paisley, Scotland). CelLytic™ B Cell Lysis Reagent (Sigma, B7435-500ML) was used for active inclusion bodies preparation.
All other reagents were of analytical grade from Sigma-Aldrich (St. Louis, MO).

Cloning, expression and isolation of active inclusion bodies

SPO0224; SPO1256; SPO1727; b0910 and Hd0053 genes were amplified from genomic DNA in 50 μL PCR reaction using forward
5´GACGACGACAAGTTGACCCATGAATCCGAC3´;
5´GACGACGACAAGTTGGAGACAGCAAAGCCC3´;
5´GACGACGACAAGTTGAACCGGAACGGCAGC3´;
5´GACGACGACAAGTTGACGGCAATTGCCCCG3';
5´GACGACGACAAGTTGCTGATTCAACAAAATCTTG3'
and reverse
5´GAGGAGAAGCCCGGTTAATAGACCTTGGGAACGTA3´;
5´GAGGAGAAGCCCGGTTAGTCCTGCCTGGCCCGCTG3´;
5´GAGGAGAAGCCCGGTTACGCATCCCAGATGTC3´;
5´GAGGAGAAGCCCGGTTATGCGAGAGCCAATTT3';
5´GAGGAGAAGCCCGGTTAATTATGTATTGTACACAT3'
primers.