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PCR and cloning

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Protocol status: Working

We use this protocol and it's working

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Materials

MATERIALS



TOPO[®] TA Cloning[®] Kit, with pCR[®]2.1-TOPO[®], One Shot TOP10 Chemically Competent E. coli, and PureLink[®] Quick Plasmid Miniprep Kit **Thermo Fisher Catalog #K450002**

Amplification of DNA was performed by two set of published hexon gene primers, H1/H2 and H3/H4 [Raue and Hess, 1998] and fiber gene primer, FibF/FibR, FibF: 5'-GGTCTACCCCTTTTGGCTCC-3' and FibR: 5'-GCGTCGTAGATGAAGGGAGG-3' [Norfitriah et al., 2018] according to manufacture protocol (Bioline, UK). The PCR products were analyzed by electrophoresis in a 1% agarose gel stained with RedSafe[™] Nuclei Acid Staining solution (iNtRON, Korea) at 70 volts for 45 minutes and visualized under U.V. transillumination. Purification of PCR products were carried out by using MEGAquick-spin[™] Total Fragment DNA Purification kit (iNtRON) based on the manufacture recommendation. Purified PCR products from H1/H2 and FibF/FibR were cloned into the pCR[™] 2.1-TOPO[®] vector using TOPO TA Cloning kit (Invitrogen, USA). Positive cloned was analyzed by colony-PCR prior plasmid extraction using DNA-spin[™] Plasmid DNA Purification kit (iNtRON) and stored at -20°C until used.

