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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.

Troubleshooting