PCR analysis of genomic DNA

Genomic DNA from *E. coli* MG1655 (Coli Genetic Stock Centre), DH5a (NEB) and Top10 (Invitrogen) was extracted using a Genomic DNA Purification Kit (ThermoFisher).
To confirm the presence/absence of the fimbriae operon, primers were designed to amplify fragments from the *fimA*, *fimD* and *fimH* coding sequences, Table 1.

CDS	**Forward primer 5'-3'**	**Reverse primer 5'-3'**	**Product size**
fimA	CTCTGGCAATCGTTGTTCTG	GAAGGTCGCATCCGCATTAG	518 bp
fimD	TCCACACTTCCCGATGAC	CACCGCTGACTCCGTAATAG	763 bp
fimH	GATGGGCTGGTCGGTAAATG	AGTCCCTACTGCTCCTAACG	763 bp

Table 1. List of primer sequences and the expected amplified product length.

Polymerase chain reactions (PCR) were set up as followed, using GoTaq DNA polymerase (Promega).

	1 rxn	Master mix for 4 rxn
5 x Green GoTaq reaction buffer	4 µl	12 µl
2 mM nucleotide mix	2 µl	8 µl
10 µM forward primer	2 µl	8 µl
10 µM reverse primer	2 µl	8 µl
5U/µl GoTaq DNA polymerase	0.1 µl	0.4 µl
10 ng/µl Genomic DNA	1 µl	
Nuclease free water	8.9 µl	35.6 µl

The PCR thermocycler was set up as follows:

Initial denaturation	95 °C	2 min
Denaturation	95 °C	30 s
Anneal	58 °C	30 s
Extension	72 °C	1 min
No. cycles	25	
Final extension	72 °C	5 min
Hold	10 °C	

PCR products were separated on a 1 % agarose gel and stained with SYBR Green for visualization.